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FORM PTO-1390 (REV. 1-93) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
471-162P	09/600991 NEW

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/00478	January 27, 1999	January 30, 1998

TITLE OF INVENTION

RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

APPLICANT(S) FOR DO/EO/US

MEDICO, Enzo; MICHIELI, Paolo; COLLESI, Chiara; CASELLI, Gianfranco; COMOGLIO, Paolo

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

JUL 26 2000 PATENT & TRADEMARK OFF.

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(3)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.-1449 and International Search Report (PCT/ISA/210) w/ 6 references

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [X] Other items or information:
    1.) Nineteen (19) sheets of Formal Drawings
    2.) Sequence Listing (8 pages)
    3.) International Preliminary Examination Report w/ 4 sheets amended claims
    4.) PCT Request (PCT/RO/101)

U.S. APPLICATION NO (if known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
NEW 09/600991	PCT/EP99/00478	471-162P

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17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. **$970.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	18 - 20 =	0	X $18.00	$ 0	
Independent Claims	1 - 3 =	0	X $78.00	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	Yes		+ $260.00	$ 260.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1230.00	
Reduction of ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 0	
SUBTOTAL =				$ 1230.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0	
TOTAL NATIONAL FEE =				$ 1230.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0	
TOTAL FEES ENCLOSED =				$ 1230.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ **1230.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account. No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:
Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292
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SIGNATURE

STEWART, RAYMOND C.
NAME

#32,881

#21,066 (RCS)
REGISTRATION NUMBER

/cqc July 26, 2000

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	MEDICO, Enzo et al.		
Int'l. Appl. No.:	PCT/EP99/00478		
Appl. No.:	New	Group:	
Filed:	July 26, 2000	Examiner:	
For:	RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP		

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

July 26, 2000

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/EP99/00478 which has an International filing date of January 27, 1999, which designated the United States of America.--

IN THE CLAIMS:

Please amend the claims as follows:

Claim 2: Line 1, change "claims 1-2" to --claim 1--

Claim 3: Line 1, change "claims 1-2" to --claim 1 or 2--

Claim 4: Line 1, change "claims 1-2" to --claim 1 or 2--

Claim 5: Line 2, change "1-5" to --1-2--

Claim 8: Line 1, change "claims 1-4" to --claim 1--

Claim 10: Line 1, change "claims 1-4" to --claim 1--

Claim 11: Line 1, change "claims 1-4" to --claim 1--

Claim 13: Line 2, change "claims 1-4" to --claim 1--

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims were also amended to delete improper multiple claims and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are respectfully requested.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____ #32,881
Raymond C. Stewart, #21,066

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RCS/cqc
471-162P

(Rev. 04/19/2000)

Applicant or Patentee: MEDICO, Enzo et al.
Serial or Patent No. 09/600,991
Filed or Issued: July 26, 2000
For: RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

Attorney's Docket No.: 0471-0162P

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am
[ ] the owner of the small business concern identified below:
[ ] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN DOMPE' S.p.A.
ADDRESS OF CONCERN Via Campo di Pile
L'AQUILA, Italy

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled Recombinant proteins derived from HGF and MSP by Enzo MEDICO, Paolo MICHIELI, Chiara COLLESI, Gianfranco CASELLI, Paolo COMOGLIO
inventor(s)

described in
[ ] the specification filed herewith
[ ] application Serial No. _____, filed _____
[ ] Patent No. _____ issued _____.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a non-profit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME _____
ADDRESS _____

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

NAME _____
ADDRESS _____

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Sergio Dompé
TITLE OF PERSON OTHER THAN OWNER Managing Director
ADDRESS OF PERSON SIGNING Via Campo di Pile, L'AQUILA, Italy

SIGNATURE _____ DATE 27.07.2000

RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

Field of the invention

The present invention relates to recombinant proteins obtained from the combination of structural domains derived from the α subunits of hepatocyte growth factor (HGF) and macrophage stimulating protein (MSP).

In particular, the engineered factors of the invention are obtained by combination of the hairpin loop and kringle domains of the α chains of HGF and/or MSP, so as to obtain a structure having two superdomains with an intervening linker sequence. Moreover, the invention relates to DNA sequences encoding the above mentioned recombinant proteins, to the expression vectors comprising said DNA sequences and to host cells containing said expression vectors. The recombinant proteins of the present invention are biologically active, and their activity can be measured by determination of their ability to induce activation of the Met tyrosine kinase receptor, their "scattering" effect on epithelial cells, and their protective effect against cell death induced by chemotherapic drugs (vide infra). Therefore, these molecules can conveniently be used to prevent or treat the toxic side effects of the chemotherapeutical treatment of tumours, and to reduce iatrogenic cell damage induced by other types of drugs.

Technological background

Hepatocyte Growth Factor (HGF) and Macrophage Stimulating Protein (MSP) are highly related proteins both structurally and

functionally (Fig. 1 and 2). Both these factors are secreted as an inactive precursor, which is processed by specific proteases which recognise a cleavage site inside the molecule, dividing the protein in two subunits. These subunits, named α chain and β chain, are linked by a disulphide bond. Thus, the mature factor is an α-β dimeric protein. Only the mature (dimeric) form of the factor is able to activate its receptor at the surface of the target cells (the Met tyrosine kinase in the case of HGF and the Ron tyrosine kinase in the case of MSP) and therefore to mediate biological responses (Naldini, L. et al., 1992, EMBO J. 11: 4825-4833; Wang, M. et al., 1994, J. Biol. Chem. 269; 3436-3440; Bottaro, D. et al., 1991, Science 25: 802-804; Naldini, L. et al., 1991, EMBO J. 10: 2867-2878; Wang, M. et al., 1994, Science 266: 117-119; Gaudino, G. et al., 1994, EMBO J. 13: 3524-3532).

The α chain of both factors contains a hairpin loop (HL) structure and four domains with a tangle-like structure named kringles (K1-K4; Nakamura T et al., 1989, Nature 342:440-443; Han, S. et al.., 1991, Biochemistry 30: 9768-9780). The precursor also contains a signal sequence (LS) of 31 amino acids (in the case of HGF) or of 18 amino acids (in the case of MSP), removed in rough endoplasmic reticulum, which directs the neoformed peptide to the secretive pathway. The β chain contains a box with a sequence homologous to that typical of serine proteases, but it has no catalytic activity (Nakamura T et al., 1989, Nature 342:440-443; Han, S. et al.., 1991, Biochemistry 30: 9768-9780). Both α and β chains contribute to the binding of the growth factor to the respective receptor (Met for HGF and

Ron for MSP).

HGF and MSP polypeptides are able to induce a variety of biological effects besides cell proliferation. The main biological activities of these molecules are: stimulation of cell division (mitogenesis); stimulation of motility (scattering); induction of polarisation and cell differentiation; induction of tubule formation (branched morphogenesis); increase of cell survival (protection from apoptosis). The tissues that respond to HGF and MSP stimulation are those where cells express the respective Met (HGF) and Ron (MSP) receptors. The most important target tissues of these factors are epithelial cells of different organs, such as liver, kidney, lung, breast, pancreas and stomach, and some cells of the hematopoietic and nervous systems. A detailed review of the biological effects of HGF and MSP in the various tissues can be found in Tamagnone, L. & Comoglio, P., 1997, Cytokine & Growth Factor Re-views, 8: 129-142, Elsevier Science Ltd.; Zarnegar, R. & Michalopoulos, G., 1995, J. Cell Biol. 129: 1177-1180; Medico, E. et al., 1996, Mol. Biol. Cell, 7: 495-504; Banu, N. et al., 1996, J. Immunol. 156: S2933-2940.

In the case of HGF, the hairpin loop and the first two kringles are known to contain the sites of direct interaction with the Met receptor (Lokker NA et al., 1992, EMBO J., 11:2503-2510; Lokker, N. et al., 1994, Protein Engineering 7: 895-903). Two naturally-occurring truncated forms of HGF produced by some cells by alternative splicing have been described. The first one comprises the first kringle (NK1-HGF Cioce, V. et al., 1996, J. Biol. Chem.

271: 13110-13115) whereas the second one spans to the second kringle (NK2-HGF Miyazawa, K. et al., 1991, Eur. J. Biochem. 197: 15-22). NK2-HGF induces cell scattering, but it is not mitogenic as the complete growth factor is (Hartmann, G. et al., 1992, Proc. Natl. Acad. Sci. USA 89: 11574-11578). However, NK2-HGF regains mitogenic activity in the presence of heparin, a glucosaminoglycan that binds HGF through a domain contained in the first kringle and which is likely to induce dimerization of NK2-HGF (Schwall, R. et al., 1996, J. Cell Biol. 133: 709-718). Moreover NK2-HGF, being a partial agonist of Met, behaves as a competitive inhibitor of HGF as far as the mitogenic activity is concerned (Chan, A. et al., 1991, Science 254: 1382-1385). NK1-HGF has also been described to exert partial stimulation of Met and competitive inhibition of HGF mitogenic activity (Cioce, V. et al., 1996, J. Biol. Chem. 271: 13110-13115). Anyway, a truncated factor is endowed with an activity markedly lower than the recombinant factors described in the invention, as shown in example 3.

In the case of MSP, the interaction sites with the Ron receptor are less understood: some preliminary studies suggest a situation opposite of that of HGF, i.e. the β chain directly binds the receptor whereas the α chain would act stabilizing the complex (Wang MH et al., 1997, J. Biol. Chem. 272:16999-17004).

The therapeutical use of molecules such as HGF and MSP is potentially valuable in a wide range of pathologies (Abdulla, S., 1997, Mol. Med. Today 3: 233). Nevertheless, a number of technical

as well as biological complications make the application of these molecules in clinics difficult. First of all, the pleiotropic character of these factors can causes poorly selective biological responses, which involve undesired side effects. For example, the use of HGF to prevent some side effects of the chemotherapeutic drug cisplatin has been proposed (Kawaida K et al., 1994, Proc. Natl. Acad. Sci. 91:4357-4361). Cancer patients treated with this drug can suffer kidney acute damage due to the cytotoxic action of cisplatin on proximal tubule epithelial cells. HGF is able to protect these cells against programmed death (apoptosis) induced by cisplatin, but at the same time it can induce an undesired proliferation of neoplastic cells. Other problems related to the pharmaceutical use of HGF and MSP are the necessity of their proteolytic activation and their stability, which causes technical problems. The NK1 and NK2 truncated forms of HGF do not require proteolytic activation, but they have a reduced biological activity.

Summary of the invention

The present invention provides recombinant molecules composed of a combination of structural domains derived from the α chains of HGF and/or MSP, which overcome the problems of the prior art molecules described above. The molecules of this invention are composed of two superdomains connected by a linker. Each superdomain is composed of a combination of the HL and K1-K4 domains of the α chain of HGF and/or MSP. These engineered factors induce selective biological responses, do not require

proteolytic activation, are stable and are more active than the truncated forms of HGF described previously.

Detailed disclosure of the invention

The present invention relates to recombinant proteins (which will be hereinafter referred to indifferently as proteins, molecules, engineered or recombinant factors) characterised by a structure that comprises two superdomains, each consisting of a combination of HL and K1-K4 domains derived from the α chain of HGF and/or MSP, linked by a spacer sequence or a linker. In particular, the invention relates to proteins of general formula (I)

$$[A] - B - [C] - (D)_y \qquad (I)$$

in which

[A] corresponds to the sequence $(LS)_m$-HL-K1-$(K2)_n$-$(K3)_o$-$(K4)_p$

wherein (the numbering of the following amino acids refers to the HGF and MSP sequences as reported in Fig. 1 and 2, respectively):

LS is an amino acid sequence corresponding to residues 1-31 of HGF or 1-18 of MSP;

HL is an amino acid sequence derived from the α chain of HGF starting between residues 32-70 and ending between residues 96-127; or it is an amino acid sequence derived from the α chain of MSP starting between residues 19-56 and ending between residues 78-109;

K1 is an amino acid sequence derived from the α chain of HGF starting between residues 97-128 and ending between residues 201-205; or it is an amino acid sequence derived from the α chain of MSP starting between residues 79-110 and ending between residues 186-190;

K2 is an amino acid sequence derived from the α chain of HGF starting between residues 202-206 and ending between residues 283-299; or it is an amino acid sequence derived from the α chain of MSP starting between residues 187-191 and ending between residues 268-282;

K3 is an amino acid sequence derived from the α chain of HGF starting between residues 284-300 and ending between residues 378-385; or it is an amino acid sequence derived from the α chain of MSP starting between residues 269-283 and ending between residues 361-369;

K4 is an amino acid sequence derived from the α chain of HGF starting between residues 379-386 and ending between residues 464-487; or it is an amino acid sequence derived from the α chain of MSP starting between residues 362-370 and ending between residues 448-481;

m, n, o, p can be 0 or 1;

the sum $n + o + p$ is an integer from 1 to 3 or 0, with the proviso that $n \geq o \geq p$;

B is the sequence $[(X)_q\ Y]_r$, wherein X = Gly and Y = Ser, or Cys, or Met, or Ala;

q is an integer from 2 to 8;

r is an integer from 1 to 9;

[C] corresponds to the sequence HL-K1-$(K2)_s$-$(K3)_t$-$(K4)_u$

wherein HL, K1-K4 are as defined above,

s, t, u are 0 or 1; the sum $s + t + u$ is an integer from 1 to 3 or 0, with the proviso that $s \geq t \geq u$;

D is the sequence W-Z, wherein W is a conventional proteolytic site, Z is any tag sequence useful for the purification and detection of the protein; y

is 0 or 1.

Non-limiting examples of W are consensus sequences for enterokinase protease, thrombin, factor Xa and IgA protease.

Preferred proteins of general formula (I), are those in which: the HL domain is a sequence of HGF α chain ranging from amino acids 32 to 127, or a sequence of MPS α chain ranging from amino acids 19 to 98; the K1 domain is a sequence of HGF α chain ranging from amino acids 128 to 203, or a sequence of MPS α chain ranging from amino acids 99 to 188; the K2 domain is a sequence of HGF α chain ranging from amino acids 204 to 294, or a sequence of MPS α chain ranging from amino acids 189 to 274; the K3 domain is a sequence of HGF α chain ranging from amino acids 286 to 383, or a sequence of MPS α chain ranging from amino acids 275 and 367; the K4 domain is a sequence of HGF α chain ranging from amino acids 384 to 487, or a sequence of MPS α chain ranging from amino acids 368 and 477.

Among the possible combinations of the domains of general formula (I), the following (II) and (III) are preferred, concerning two recombinant factors named Metron Factor-1 and Magic Factor-1, respectively:

LS_{MSP}-HL_{MSP}-$K1_{MSP}$-$K2_{MSP}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (Metron Factor-1) (II)

and

LS_{HGF}-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (Magic Factor-1) (III)

For both molecules, L is a linker sequence $(Gly_4Ser)_3$, D is a tag

sequence Asp_4-Lys-His_6.

For Metron Factor-1, LS_{MSP} is the sequence 1-18 of MSP, HL_{MSP} is the sequence 19-56 of MSP, $K1_{MSP}$ is the sequence 99-188 of MSP, $K2_{MSP}$ is the sequence 189-274 of MSP, HL_{HGF} is the sequence 32-127 of HGF, $K1_{HGF}$ is the sequence 128-203 of HGF, $K2_{HGF}$ is the sequence 204-294 of HGF.

For Magic Factor-1, HL_{HGF}, $K1_{HGF}$, $K2_{HGF}$ are as defined above, LS_{HGF} is the sequence 1-31 of HGF.

The hybrid molecules of the invention are prepared by genetic engineering techniques according to a strategy involving the following steps:

a) construction of DNA encoding the desired protein;

b) insertion of DNA in an expression vector;

c) transformation of a host cell with recombinant DNA (rDNA);

d) culture of the transformed host cell so as to express the recombinant protein;

e) extraction and purification of the produced recombinant protein.

The DNA sequences corresponding to HGF or MSP structural domains can be obtained by synthesis or starting from DNA encoding for the two natural factors. For example, screening of cDNA libraries can be carried out using suitable probes, so as to isolate HGF or MSP cDNA. Alternatively, HGF or MSP cDNA can be obtained by reverse transcription from purified mRNA from suitable cells.

cDNAs coding for the fragments of HGF and MSP β chains can be amplificated by PCR (Mullis, K.B. and Faloona, F.A., 1987, Methods in

Enzymol. 155, 335-350), and the amplification products can be recombined making use of suitable restriction sites, naturally occurring in the factor sequences or artificially introduced in the oligonucleotide sequence used for the amplification.

In greater detail, one of the above mentioned strategies can be the following:

the portions of DNA encoding the LS, HL, K1, K2, K3 and K4 domains are amplificated by PCR from HGF or MSP cDNA and then recombined to obtain the hybrid sequences corresponding to [A] and [C]. Oligonucleotides recognising sequences located at the two ends of the domains to be amplificated are used as primers. Primers are designed so as to contain a sequence allowing recombination between the DNA of a domain and the adjacent one. Said recombination can be carried out by endonuclease cleavage and subsequent ligase reaction, or making use of the recombinant PCR method (Innis, NA et al.., 1990, in PCR Protocols, Academic Press, 177-183).

The sequence encoding the domain B (linker) can be obtained by synthesis of a double chain oligonucleotide, which can be inserted between [A] and [C] using suitable restriction sites.

The resulting three fragments encoding for [A], [B] and [C] are then inserted in the correct sequence in a suitable vector. In this step it can be decided whether to add or not the domain D (tag), obtained by synthesis analogously to domain B, downstream fragment [C].

The recombinant expression vector can contain, in addition to the recombinant construct, a promoter, a ribosome binding site, an initiation

codon, a stop codon, optionally a consensus site for expression enhancers.

The vector can also comprise a selection marker for isolating the host cells containing the DNA construct. Yeast or bacteria plasmids, such as plasmids suitable for Escherichia Coli, can be used as vectors, as well as bacteriophages, viruses, retroviruses, or DNA.

The vectors are cloned preferably in bacterial cells, for example in Escherichia Coli, as described in Sambrook J., 1989, Molecular Cloning, Cold Spring Harbor Laboratory Press, New York, and the colonies can be selected, for example, by hybridisation with radiolabelled oligonucleotide probes; subsequently, the rDNA sequence extracted from the positive colonies is determined by known methods.

The vector with the recombinant construct can be introduced in the host cell according to the competent cell method, the protoplast method, the calcium phosphate method, the DEAE-dextran method, the electric impulses method, the in vitro packaging method, the viral vector method, the micro-injection method, or other suitable techniques.

Host cells can be prokaryotic or eukaryotic, such as bacteria, yeasts or mammal cells, and they will be such as to effectively produce the recombinant protein.

After transformation, cells are grown in a suitable medium, which can be for example MEM, DMEM or RPMI 1640 in the case of mammal host cells.

The recombinant protein is secreted in the culture medium from which it can be recovered and purified with different methods, such as mass exclusion, absorption, affinity chromatography, salting-out, precipitation, dialysis, ultrafiltration.

A simple, rapid system for the production of the molecules of the invention is, for example, transient expression in mammal cells.

Accordingly, the plasmid containing the recombinant DNA fragment, for example PMT2 (Sambrook, J. et al., 1989, Molecular Cloning, Cold Spring Harbor Laboratory Press), is transfected in suitable recipient cells, such as Cos7 (Sambrook, J. et al., supra) by the calcium phosphate technique or other equivalent techniques. Some days after transfection, the conditioned medium of the transfected cells is collected, cleared by centrifugation and analysed for its content in factor. For this analysis, antibodies directed against HGF or MSP, or against any tag sequence, can be used: the supernatant is immunoprecipitated and then analysed by western blot with the same antibody. The supernatant containing the recombinant factor can also be used directly for biochemical and biological tests. The protein can be purified, for example, using a poly-histidine tag sequence, by absorption on a nickel resin column and subsequent elution with imidazole.

The biochemical properties of the recombinant factors of the invention were tested in connection with their ability to activate Met and Ron receptors.

Sub-micromolar concentrations of the factors have proved to induce phosphorylation in Met tyrosine in human epithelial cells A549, whereas

they do not induce phosphorylation above basal values in cells expressing Ron. On the whole, the tests proved that the first two kringles of HGF maintain their ability to interact and to activate Met tyrosine kinase receptor, whereas the corresponding first two kringles of MSP are not sufficient for modulating the catalytic activity of the Ron receptor. However, the interaction with Ron, although at low affinity, can contribute to the recruitment of the factor at the cell surface, playing a similar role to low affinity receptors (of mature glycoprotein) which recruit the HGF intact molecule through the heparin-binding domain.

The molecules of the invention have a marked biological activity, measured by the scattering tests, and a protecting activity against cell apoptosis induced by cisplatin or etoposide.

In particular, the supernatant containing the recombinant factor has been found to promote scattering of epithelial cells of various nature even at nanomolar concentrations. In these tests, kidney epithelial cells (MDCK) or hepatocyte precursors (MLP29) were used.

In an in vitro experimental system, in which DNA fragmentation typical of apoptotic cells is evaluated by the TUNEL method (Gavrieli, Y. et al., 1992, J. Cell. Biol. 117, 493-501), the recombinant factors protect against apoptosis induced by chemotherapeutic drugs at levels comparable with HGF and remarkably higher than MSP. The engineered molecules proved to be active on human primary epithelial cells from proximal tubule (PTECs), on an immortalised PTECs line (Loc) and on the already cited murine hepatocytes MLP29.

Among the applications of the recombinant molecules of the

invention, the following can be cited:

- prevention of myelotoxicity; in particular they can be used for the expansion of marrow precursors, to increase proliferation of the hematopoietic precursors or to stimulate their entry in circle;
- prevention of liver and kidney toxicity, and of mucositis following antineoplastic treatments; in particular the recombinant factors can be used to prevent toxicity (apoptosis) on differentiated cell elements of liver, kidney and mucosa of the gastroenteral tract, and to stimulate staminal elements of cutis and mucosas to allow the regeneration of germinative layers;
- prevention of chemotherapeutic neurotoxicity.

In general, the proteins of the invention provide the following advantages, compared with the parent molecules HGF and MSP:

- they are smaller molecules with a more compact structure;
- they are more stable and are produced in higher amounts;
- they require no endoproteolytic cleavage for activation, which transforms the HGF and MSP precursors into the respective active forms;
- they can be engineered in combinations of different functional domains, thereby modulating the biological effects, increasing the favourable ones and reducing those undesired (for example, protection from apoptosis versus cell proliferation).

The invention has to be considered also directed at amino acid and nucleotide sequences referred to formula (I), having modifications which can, for example, derive from degeneration of genetic code, without

therefore modifying the amino acid sequence, or from the deletion, substitution, insertion, inversion or addition of nucleotides and/or bases according to all the possible methods known in the art.

Furthermore, the invention relates to the expression vectors comprising a sequence encoding for a protein of general formula (I), which can be plasmids, bacteriophages, viruses, retroviruses, or others, and to host cells containing said expression vectors.

Finally, the invention relates to the use of the recombinant proteins as therapeutical agents, and to pharmaceutical compositions containing an effective amount of the recombinant proteins together with pharmacologically acceptable excipients.

Description of the Figures

(In the following legends, -His located after the name of the parent factors, truncated or recombinant, or of the plasmids, means that the respective sequences contain a poly-histidine tag).

Figure 1:

a) Nucleotide and amino acid sequence of human HGF (Gene Bank # M73239; Weidner, K.M., et al., 1991, Proc. Acad. Sci. USA, 88:7001-7005). In contrast to the cited reference, in the numbering used herein, nucleotide No. 1 is the first base of the initiation codon (the A of the first ATG). The first amino acid is the corresponding methionine. The cDNA untranslated regions at 5' and 3' are neither represented nor considered in the numbering.

b) Nucleotide and amino acid sequence of human MSP (Gene Bank # L11924; Yoshimura, T., et al., 1993, J. Biol. Chem., 268:15461-

15468). In contrast to the cited reference, in the numbering used herein nucleotide No. 1 is the first base of the initiation codon (the A of the first ATG). The first amino acid is the corresponding methionine. The cDNA untranslated regions at 5' and 3' are neither represented nor considered in the numbering.

Figure 2:

a) Molecular structure of Metron Factor-1. The leader sequence is removed from the cells used for the production before secretion and is therefore absent in the mature molecule. The poly-histidine tag can be removed by digestion with the protease enterokinase.

b) Nucleotide and amino acid sequence of Metron-Factor-1. The nucleotide sequence starts with the EcoRI site and terminates with the SalI site (first six bases and last six bases, respectively). The initiation codon (ATG) and the stop codon (TAG) are underlined.

Figure 3:

a) Molecular structure of Magic Factor-1. The leader sequence is removed from the cells used for the production before secretion and is therefore absent in the mature molecule. Poly-histidine tag can be removed by digestion with the protease enterokinase.

b) Nucleotide and amino acid sequence of Magic Factor-1. The nucleotide sequence starts with the SalI site (first six bases and last six bases, respectively). The initiation codon (ATG) and the stop codon (TAG) are underlined.

Figure 4:

Production of Metron-F-1 by transient transfection of mammal

cells. The conditioned supernatants from BOSC cells transfected with the control plasmid (CTRL) or with pRK7-Metron F-1-His were immunoprecipitated with an anti-MSP polyclonal antibody and detected by western blot with the same antibody.

Figure 5:

Quantitation of the recombinant proteins by western blot. (A) The proteins were absorbed on Sepharose-A-heparin beads and detected with an anti-poly-histidine monoclonal antibody. (B) The proteins were immunoprecipitated with an anti-MSP polyclonal antibody and detected with an anti-poly-histidine monoclonal antibody.

Figure 6:

Scattering test carried out on kidney epithelial cells (MDCK) using the recombinant proteins prepared by transient transfection. The protein content was quantified by western blot (see Fig. 5). (A) non-stimulated cells; (B) cells stimulated with control supernatant; (C) cells stimulated with HGF-His; (D) cells stimulated with NK2-HGF-His; (E) cells stimulated with Metron Factor-1; (F) cells stimulated with Magic Factor-1.

Figure 7:

Activation (phosphorylation) of Met receptor by the hybrid factor Metron Factor-1. Human epithelial cells (A549) were stimulated with supernatants conditioned from BOSC cells transfected with the control plasmid (CTRL) or with pRK7-Metron-F-1-His (METRON F-1) at the indicated dilutions. Cell lysates from the stimulated cells were immunoprecipitated with an anti-Met monoclonal antibody and detected by western blot with an anti-phosphotyrosine monoclonal antibody.

Figure 8:

Protective effect of Metron-F-1 against acute renal failure induced by $HgCl_2$ in vivo. Balb-c mice were injected i.v. with Metron-F-1 or vehicle at 0.5 h before and 6, 12, 24, 36 and 48 h after $HgCl_2$ i.v. administration. BUN and histological evaluation of renal necrosis were measured at 72 h.

Data expressed as mean + e.s. of 7 animals/group (BUN) or 3 animals/group (histology).

The following examples illustrate in greater detail the invention.

Example 1a: Preparation of the recombinant construct encoding Metron Factor-1

HGF cDNA was obtained by the RT-PCR technique (Reverse Transcriptase PCR; in: Innis, M. A., et al.., 1990, PCR Protocols, Academic Press, 21-27) from a human lung fibroblast cell line (MRC5; Naldini, L. et al., 1991, EMBO J. 10: 2867-2878). MSP cDNA was obtained with the same technique from human liver (Gaudino, G., et al., 1994, EMBO J. 13: 3524-3532).

The fragment corresponding to MSP LS-HL-K1-K2 was amplified by PCR using MSP cDNA as template and the following oligonucleotides as primers:

P1 (sense)

5' CGCGCGGAATTCCACCATGGGGTGGCTCCCACTCCT 3'

P2 (antisense)

5' CGCGCGCTCGAGGCGGGGCTGTGCCTCGGACCCGCA 3'

in which the underlined palindromic sequences are the restriction sites for

the enzymes EcoRI (oligonucleotide P1) and XhoI (oligonucleotide P2). The PCR product was digested with the restriction enzymes EcoRI and XhoI and then purified by electrophoresis on agarose gel.

The fragment corresponding to HL-K1-K2 of HGF was amplified by PCR using HGF cDNA as template and the following oligonucleotides as primers:

P3 (sense)

5' CGCGCGTCTAGAGGGACAAAGGAAAAGAAGAAATAC 3'

P4 (antisense)

5' CGCGCGAAGCTTTGTCAGCGCATGTTTTAATTGCAC 3'

in which the underlined palindromic sequences are the restriction sites for the enzymes XbaI (oligonucleotide P3) and HindIII (oligonucleotide P4). The PCR product was digested with the restriction enzymes XbaI and HindIII and then purified by electrophoresis on agarose gel.

For the linker sequence, the following partially complementary oligonucleotides were synthesised, and were subsequently annealed to obtain a double strand DNA fragment with sticky ends:

P5 (sense)

5'TCGAGGGCGGTGGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCT 3'

P6 (antisense)

5'CTAGAGAACCGCCACCGCCGGAGCCACCGCCACCAGAACCGCCACCGCCC 3'

in which the underlined bases are the sequences compatible with the restriction sites for the enzymes XhoI (oligonucleotide P5) and XbaI (oligonucleotide P6).

The resulting three DNA fragments were subcloned in the EcoRI-

HindIII sites of the expression vector pRK7 (Gaudino, G., et al., 1994, EMBO J. 13: 3524-3532), to obtain the recombinant plasmid pRK7-Metron-F-1, containing all the components of Metron Factor-1 except the tag sequence.

For the insertion of the tag sequence, the following partially complementary oligonucleotides were synthesised, and were subsequently annealed to obtain a double strand DNA fragment with sticky ends:

P7 (sense)

5'AGCTGACGACGACGACAAACACCACCACCACCACCACTAGGGTCGAC3'

P8 (antisense)

5'AGCTGTCGACCCTAGTGGTGGTGGTGGTGGTGTTTGTCGTCGTCGTC3'

in which the underlined bases are compatible with the HindIII restriction site and the boxed palindromic sequences are the consensus sequences for the enzyme SalI. The resulting double strand DNA fragment was inserted in the restriction site HindIII of the recombinant plasmid obtained at the previous step (destroying the HindIII site and creating the SalI site), to obtain the plasmid pRK7-Metron-F-1-His.

Example 1b: Production of Metron Factor-1

The expression vector pRK7 contains a promoter of human cytomegalovirus immediate-early gene (CMV) and an episomal replication origin site of the DNA virus SV40. Therefore, this plasmid is particularly suitable for the expression of proteins in cells expressing the large T antigen of the virus SV40, such as kidney epithelial BOSC cells (Sambrook, J. et al.., 1989, Molecular Cloning, Cold Spring Harbor Laboratory Press). Metron Factor-1 can then be produced by transient

transfection of plasmid pRK7-Metron F-1-His in BOSC cells.

For transfection, 10^6 cells are seeded at day 0 in a 100 mm plate in 90% Dulbecco's Modified Eagle Medium (DMEM)-10% bovine calf serum (10 ml/plate). At day 1, cells are transfected with 10 µg/plate of pRK7-Metron-F-1-His by lipofection, using the protocol provided by the lipofectin producer (Gibco-BRL). At day 2, the DNA-containing medium is substituted by fresh medium with low content in serum (99.5% DMEM-0.5% bovine calf serum). At day 4 (48 hours after the end of the transfection), the medium is collected, cleared by centrifugation, and analysed for its content in Metron Factor-1.

This analysis can be carried out in different ways. For example, the recombinant protein present in the cleared supernatant can be immunoprecipitated with an anti-MSP antibody and then detected by western blot with the same antibody (Fig. 4). In the example shown in figure 4, 500 µl of supernatant (cleared by centrifugation, buffered in 25 mM HEPES and added with a protease inhibitors cocktail) were immunoprecipitated (2 hours at 4° C) with 20 µl of Sepharose-A beads (Pharmacia) covalently conjugated with 2 µl of anti-MSP polyclonal antibody. The beads pellet was washed 3 times with 500 µl of washing buffer (20 mM HEPES pH 7.4; 150 mM NaCl; 0.1% Triton X-100; 10% glycerol) and heated at 90° C for 2 minutes in 100 µl of Laemmli buffer. Eluted proteins were separated by SDS-PAGE on 8% BIS-acrylamide gel, transferred onto membrane (Hybond-C; Amersham) and analysed by western blot. For this analysis, the same rabbit serum used for immunoprecipitation was employed as primary antibody with a 1:1000

dilution and protein A conjugated with peroxidase (Amersham) was used as secondary antibody. Protein A was detected by ECL (Amersham) following the protocol provided by the producer.

Alternatively, the recombinant protein can be partially purified by adsorption on Sepharose-A beads conjugated with heparin and subsequent analysis by western blot using antibodies directed to poly-histidine tag (Fig. 5).

In the example shown in figure 5, the Sepharose-A-heparin beads (20 µl; Pierce) were incubated (4 hours at 4° C) with 500 µl of supernatant (cleared by centrifugation, buffered in 25 mM HEPES and added with a protease inhibitors cocktail) in the presence of 500 mM NaCl, washed with suitable buffer (500 mM NaCl; 20 mM HEPES pH 7.4; 0.1% Triton X-100; 10% glycerol) and heated at 90° C for 2 minutes in 100 µl Laemmli buffer. Eluted proteins were separated by SDS-PAGE on 8% bis-acrylamide gel, transferred onto membrane (Hybond-C; Amersham) and analysed by western blot. For this analysis, a mouse monoclonal antibody to poly-histidine (Invitrogen) diluted 1:5000 was used as primary antibody and an anti-mouse IgG ovine antibody conjugated with peroxidase (Amersham) was used as secondary antibody. The secondary antibody was detected by ECL (Amersham) following the protocol provided by the producer.

The procedure of adsorption on heparin beads can also be used as protocol for the semi-purification of the recombinant protein. Furthermore, the molecule can additionally be purified making use of the poly-histidine affinity to heavy metals such as nickel. The protein containing poly-

histidine tag can be adsorbed on a nickel resin column (Invitrogen) and subsequently eluted with imidazole (the detailed protocol is provided by the manufacturer).

Example 1c: METRON-F-1 production in insect cells

The cDNA encoding for Metron-F1 was subcloned in a suitable expression vector (p-FASTBAC) to generate a recombinant plasmid containing the Metron-F1 gene (p-FASTBAC-Metron). A competent E. Coli strain (DH10 Bac) was transformed with p-FASTBAC-Metron to generate BACMID DNA. The DNA of positive colonies was isolated and checked by PCR to show the correct integration of the expression vector. Subsequently, the DNA from three clones was transfected into Sf9 insect cells with CellFECTIN reagent to produce virus particles. Virus titer was tested by a plaque assay. Single plaques were isolated and used for further propagation of the baculovirus. Viral stock was subsequently expanded in insect cells to scale up METRON-F-1 production. To verify protein expression, insect cells were infected with a multiplicity of infection (MOI) of 1 in a small-scale reactor. Samples of supernatants were analysed by SDS-PAGE followed by western blotting.

To produce amounts adequate for in vivo testing, insect cells were propagated in a 2.5-Liter stirred tank bioreactor. Cells were grown to a cell density of 1.106 ml^{-1} before they were infected with a MOI of 1. Cell suspension was harvested 3 days post infection. The supernatant containing the recombinant protein was separated by centrifugation. The presence of Metron F-1 in the supernatant was proved by SDS-PAGE followed by western blotting. Metron F-1 was pre-purified by a dual step

affinity chromatography on heparin sepharose (heparin-Hi Trap, Pharmacia) at 6° C. For in vivo testing or for further purification steps, the eluted fractions containing Metron F-1 were desalted by Sephadex G-25 chromatography (PD-10 or HiPrep 26/10, Pharmacia). Metron F-1 was further purified by chromatography on HisTrap columns (Pharmacia) and eluted by an imidazole gradient (0-0.5 M) using either a low-pressure system (Econo System, BIO-RAD) or an FPLC system (Pharmacia). Metron F-1 was eluted at an imidazole concentration of about 0.15 M. For in vivo testing, the eluted fractions containing Metron F-1 were freed of imidazole by Sephadex G-25 chromatography as already described, using the buffer to be used for animal treatment.

Example 2a: Preparation of the recombinant construct encoding for Magic Factor-1

HGF cDNA and the plasmid pRK7-Metron-F-1-His described above were used as starting DNA. The fragment corresponding to LS-HL-K1-K2 of HGF was amplificated by PCR using HGF cDNA as template and the following oligonucleotides as primers:

P9 (sense)

5' CGCGCGGGATCCGCCAGCCCGTCCAGCAGCACCATG 3'

P10 (antisense)

5' CGCGCGAAGCTTTGTCAGCGCATGTTTTAATTGCAC 3'

in which the underlined palindromic sequences are the restriction sites for the enzymes BamHI (oligonucleotide P9) and HindIII (oligonucleotide P10). The PCR product was digested with the restriction enzymes BamHI and HindIII and then purified by electrophoresis on agarose gel.

For the linker, the following partially complementary oligonucleotides were synthesized, and subsequently annealed to obtain a double strand DNA fragment with sticky ends:

P11 (sense)

5'AGCTTCGGGCGGTGGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCT3'

P12 (antisense)

5'CTAGAGAACCGCCACCGCCGGAGCCACCGCCACCAGAACCGCCACCGCCCGA3'

in which the underlined bases are the sequences compatible with the restriction sites for the enzymes HindIII (oligonucleotide P11) and XbaI (oligonucleotide P12). The fragment resulting by PCR and the double strand linker sequence were inserted in the plasmid pRK7-Metron-F-1-His in place of the fragment EcoRI-XbaI by means of an EcoRI-BamHI adapter, to obtain the plasmid pRK7-Magic-F-1-His.

Example 2b: Production of Magic Factor-1

Magic Factor-1 is produced on a small scale by transient transfection of BOSC cells analogously to what described for Metron Factor-1. Semi-purification is performed by adsorption on Sepharose-A beads conjugated with heparin followed by Western blot analysis using anti-poly-histidine antibodies (Fig. 5).

Example 3: Biological activity (scattering) on epithelial cells.

The biological activity of recombinant HGF, NK2-HGF, Metron Factor-1 and Magic Factor-1 was tested by a "scatter" assay on MDCK epithelial cells. For this functional test, cells are plated at day 0 in 96-well plates (10^3 cells/well) in 90% DMEM - 10% bovine calf serum. At day 1 the medium is substituted with fresh medium buffered with 50 mM

HEPES pH 7.4 and the supernatant containing the recombinant protein is added at different dilutions. At day 2 cells are washed with DPBS (Dulbecco's Phosphate Buffered Saline), fixed in 11% glutaraldehyde, stained with a Crystal-Violet solution and analysed by microscopy. The scattering activity is evaluated observing the morphology of the colonies, which are clustered in the negative control (non-stimulated cells or stimulated with supernatant containing no factors) whereas they are dispersed in the positive control (HGF-His). The morphology of the cells themselves also varies upon stimulation: in fact, as it can be observed in Fig. 6, cells stimulated with HGF-His and Metron Factor-1 have a more oblong, spindle-shaped form, characterised by protrusions of the cell membrane called pseudopodes. These morphological variations are the consequence of factor-induced activation of a genetic program involving the modification of a series of cellular parameters, such as digestion of cell matrix by specific proteases and increase in motility.

The Table summarises the results of different tests, obtained with factors HGF, NK2-HGF, Metron Factor-1 and Magic Factor-1 on MDCK cells. The scattering units reported indicate the maximum dilution of the conditioned supernatant containing the factor, at which motogenic activity could be observed. Values are normalised for the protein content determined by western blotting as described above (see Fig. 5). These data indicate that the hybrid factors Metron Factor-1 and Magic Factor-1 have a scattering activity approximately three magnitudes higher than that of the NK2-HGF-His truncated form and one magnitude higher than that of HGF-His parental factor.

	HGF-his	NK2-his	Metron F-1	Magic Factor-1
Scatter units	900 ± 29	6 ± 5	5500 ± 1532	7600 ± 150

Table. Scattering activity of factors HGF-His, NK2-HGF-His Metron Factor-1 measured on kidney epithelial cells (MDCK). The scattering units reported indicate the maximum dilution of the conditioned supernatant containing the factor, at which a motogenic activity can be observed. Values are normalised for the protein content determined by western blotting.

Example 4a: Test for the evaluation of protection against programmed cell death (apoptosis).

One of the most characterised side effect of the chemotherapeutic drug cisplatin is the induction of programmed cell death (apoptosis) of epithelial cells of the proximal tubule, which leads to acute renal failure (ARF). Thus, a factor that protects against cisplatin-induced cytotoxicity is highly desirable. An in vitro functional test has been used, which allows to evaluate the percentage of cisplatin-treated apoptotic cells in the presence or in the absence of a survival factor. This system utilises a cell line (LOC) derived from epithelial cells of human kidney proximal tubule, immortalised by ectopic expression of SV40 large T antigen. For the functional test, cells are plated at day 0 in 96-well plates (10^3 cells/well) in 90% DMEM - 10% bovine calf serum. At day 1, the medium is substituted with medium containing 0.5% bovine calf serum buffered with 50 mM HEPES pH 7.4, which is added with different dilutions of the supernatant containing the recombinant factor. Cells are pre-incubated with these

factors for 6 hours, and then further incubated in the presence of 10 µg/ml cisplatin. At day 2, cells are washed with DPBS and the percentage of apoptotic cells is evaluated by the TUNEL technique (Boehringer Mannheim). The same kind of tests can be performed using primary cultures of human epithelial cells of kidney proximal tubule (PTEC). These tests proved that Metron Factor-1 and Magic Factor-1 have protecting activity against cisplatin-induced programmed cell death.

Example 4b: Protection against cisplatin-induced cytotoxicity by transient gene delivery of Metron Factor-1 and Magic Factor-1

The protective effect of Metron F-1 and Magic F-1 against cisplatin-induced cytotoxicity was further demonstrated by a transient gene delivery approach. Simian kidney epithelial cells (COS) were transfected with a control empty vector, an expression vector for Metron F-1, or an expression vector for Magic F-1. Following transfection, cells were treated for 16 hours with cisplatin (20 µg/ml) and the percentage of surviving cells in each transfection was determined. Cisplatin treatment was calibrated to cause the death of approximately 20% of the cells in the negative control. Ectopic expression of Metron F-1 or Magic F-1 increased the survival rate to about 92.3% and 94.0%, respectively.

Example 5: Activation of the Met receptor by Metron Factor-1 and Magic Factor-1

The ability of Metron Factor-1 and Magic Factor-1 to activate the Met receptor was tested by analysing the ability of the recombinant factors to induce tyrosine phosphorylation of Met in human epithelial cells (A549). For this analysis, A549 cells at 90% confluence in a 100 mm petri

dish were stimulated for 10 minutes with 1 ml of conditioned supernatant containing Metron Factor-1, Magic Factor-1 or no factor (as negative control) diluted 1:2.5 or 1:10 in DMEM. After stimulation, cells were washed in ice with PBS, lysated in 200 µl of lysis solution (1% Triton X-100, 5 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl pH 7.4), added with a cocktail of protease inhibitors, immunoprecipitated for 2 hours at 4° C with 10 µl of Sepharose-A beads covalently conjugated with an anti-Met monoclonal antibody (Naldini, L. et al., 1991, EMBO J. 10: 2867-2878), washed 3 times in the same lysis solution, and heated at 90°C for 2 minutes to elute the absorbed proteins. These were separated by SDS-PAGE on a 8% BIS-acrylamide gel, transferred onto a membrane (Hybond-C; Amersham) and analysed by western blot. A mouse monoclonal antibody against phosphotyrosine (UBI) diluted 1:10000 was used as primary antibody and an anti-mouse IgG ovine antibody conjugated with peroxidase (Amersham) was used as secondary antibody. The secondary antibody was detected by ECL (Amersham) following the protocol provided by the manufacturer. This analysis revealed that Metron F-1 and Magic F-1 potently activate the Met receptor (Fig. 7).

Example 6: Protection against chemotherapy-induced renal failure by Metron Factor-1 in vivo

Metron-F-1 was tested in a model of nephrotoxicity in Balb-c mice. The method used was substantially as described (Kawaida K et al., 1994, Hepatocyte growth factor prevents acute renal failure and accelerates renal regeneration in mice, Proc. Natl. Acad. Sci. 91:4357-4361). Briefly, renal failure was induced in male Balb-c mice weighing 20-25 g by an i.v.

injection of 7.5 mg/kg of $HgCl_2$ (7 animals/group). Renal damage was assessed by analysis of Blood Urea Nitrogen (BUN) and by histological evaluation, 72 h after $HgCl_2$ injection. Metron-F-1 was dissolved in 0.2 M NaCl, containing 0.01% Tween 80 and 0.25% human serum albumin and administered i.v. (100 μg/kg in a posological volume of 6.6 ml/kg) 0.5 h before and 6, 12, 24, 36 and 48 h after $HgCl_2$ injection. Controls animals were treated with the same amount of vehicle according to the same scheme.

Metron-F-1 significantly prevented the onset of acute renal failure induced by $HgCl_2$, evaluated in terms of BUN (figure 8). BUN values were closely paralleled by the histological findings, scored by an independent investigator.

In the following sequence listing:

SEQ. ID. NO. 1: Magic F-1 DNA coding sequence;

SEQ. ID. NO. 2: Magic F-1 amino acid sequence;

SEQ. ID. NO. 3: Metron F-1 DNA coding sequence;

SEQ. ID. NO. 4: Metron F-1 amino acid sequence.

ART 34 AMDT

CLAIMS

1. Recombinant proteins comprising two superdomains, separated by a spacer sequence (linker), obtained combining the HL and K1-K4 domains of HGF and/or MSP α chains, according to general formula (I):

$$[A] - B - [C] - (D)y \qquad (I)$$

in which

[A] corresponds to the sequence $(LS)_m$-HL-K1-$(K2)_n$-$(K3)_o$-$(K4)_p$

wherein (the numbering of the following amino acids refers to the HGF and MSP sequences as reported in Fig. 1 and 2, respectively):

LS is an amino acid sequence corresponding to residues 1-31 of HGF or 1-18 of MSP;

HL is an amino acid sequence starting between residues 32-70 of HGF α chain and ending between residues 96-127 of the identical chain; or it is an amino acid sequence starting between residues 19-56 of MSP α chain and ending between residues 78-109 of the identical chain;

K1 is an amino acid sequence starting between residues 97-128 of HGF α chain and ending between residues 201-205 of the identical chain; or it is an amino acid sequence starting between residues 79-110 of MSP α chain and ending between residues 186-190 of the identical chain;

K2 is an amino acid sequence starting between residues 202-206 of HGF α chain and ending between residues 283-299 of the identical chain; or it is an amino acid sequence starting between residues 187-191 of MSP α chain and ending between residues 268-282 of the identical chain;

K3 is an amino acid sequence starting between residues 284-300 of HGF α chain and ending between residues 378-385 of the identical chain; or it is

an amino acid sequence starting between residues 269-283 of MSP α chain and ending between residues 361-369 of the identical chain;

K4 is an amino acid sequence starting between residues 379-386 of HGF α chain and ending between residues 464-487 of the identical chain; or it is an amino acid sequence starting between residues 362-370 of MSP α chain and ending between residues 448-481 of the identical chain;

m, n, o, p are 0 or 1;

the sum n + o + p is an integer from 1 to 3 or 0, with the proviso that n ≥ o ≥ p;

B is the sequence $[(X)_q\ Y]_r$, wherein X = Gly and Y = Ser, or Cys, or Met, or Ala;

q is an integer from 2 to 8;

r is an integer from 1 to 9;

[C] corresponds to the sequence HL-K1-$(K2)_s$-$(K3)_t$-$(K4)_u$

wherein HL, K1-K4 are as defined above,

s, t, u are 0 or 1; the sum s + t + u is an integer from 1 to 3 or 0, with the proviso that s ≥ t ≥ u;

D is the sequence W-Z, wherein W is a conventional proteolytic site, Z is any tag sequence useful for the purification and detection of the protein; y is 0 or 1.

2. Recombinant proteins according to claims 1-2, in which the HL domain is a sequence of HGF α chain ranging from amino acids 32 to 127, or a sequence of MPS α chain ranging from amino acids 19 to 98; the K1 domain is a sequence of HGF α chain ranging from amino acids 128 to 203, or a sequence of MPS α chain ranging from amino acids 99 to 188; the K2 domain is a sequence of HGF α chain ranging from amino acids 204 to 294,

or a sequence of MPS α chain ranging from amino acids 189 to 274; the K3 domain is a sequence of HGF α chain ranging from amino acids 286 to 383, or a sequence of MPS α chain ranging from amino acids 275 to 367; the K4 domain is a sequence of HGF α chain ranging from amino acids 384 to 487, or a sequence of MPS α chain ranging from amino acids 368 to 477.

3. Recombinant proteins according to claims 1-2 of formula (II):

LS_{MSP}-HL_{MSP}-$K1_{MSP}$-$K2_{MSP}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (II)

in which LS_{MSP} is the sequence 1-18 of MSP, HL_{MSP} is the sequence 19-56 of MSP, $K1_{MSP}$ is the sequence 99-188 of MSP, $K2_{MSP}$ is the sequence 189-274 of MSP, HL_{HGF} is the sequence 32-127 of HGF, $K1_{HGF}$ is the sequence 128-203 of HGF, $K2_{HGF}$ is the sequence 204-294 of HGF, L is the sequence $(Gly_4Ser)_3$, D is the sequence Asp_4-Lys-His_6.

4. Recombinant proteins according to claims 1-2 of formula (III):

LS_{HGF}-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (III)

in which HL_{HGF}, $K1_{HGF}$, $K2_{HGF}$, L and D are as defined in claim 4, LS_{HGF} is the sequence 1-31 of HGF.

5. Nucleotide sequences encoding for the recombinant proteins of claims 1-5.

6. Expression vectors comprising the nucleotide sequences of claim 5.

7. Prokaryotic or eukaryotic host cell transformed with the expression vector of claim 6.

8. Process for preparing the recombinant proteins of claims 1-4, which comprises the following steps:

a) construction of DNA encoding the desired protein;

b) insertion of DNA in an expression vector;

c) transformation of a host cell with recombinant DNA (rDNA);

d) culture of the transformed host cell so as to express the recombinant protein;

e) extraction and purification of the produced recombinant protein.

9. Process according to claim 8, wherein the host cell is kidney epithelial BOSC cell or SF9 insect cell.

10. Recombinant proteins of claims 1-4 for use as therapeutic agents.

11. Use of recombinant proteins of claims 1-4 in the manufacture of a medicament for the prevention or treatment of chemotherapeutic-induced toxicity.

12. Use according to claim 9, wherein the chemotherapeutic-induced toxicity is myelotoxicity, kidney toxicity, neurotoxicity, mucotoxicity and hepatotoxicity.

13. Pharmaceutical compositions containing an effective amount of the recombinant proteins of claims 1-4, in combination with pharmacologically acceptable excipients.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: DOMPE' S.p.A.
(B) STREET: Via Campo di Pile
(C) CITY: L'AQUILA
(E) COUNTRY: ITALY
(F) POSTAL CODE (ZIP): 67100

(ii) TITLE OF INVENTION: RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1725 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATGTGGGTGA CCAAACTCCT GCCAGCCCTG CTGCTGCAGC ATGTCCTCCT GCATCTCCTC 60

CTGCTCCCCA TCGCCATCCC CTATGCAGAG GGACAAAGGA AAAGAAGAAA TACAATTCAT 120

GAATTCAAAA AATCAGCAAA GACTACCCTA ATCAAAATAG ATCCAGCACT GAAGATAAAA 180

ACCAAAAAAG TGAATACTGC AGACCAATGT GCTAATAGAT GTACTAGGAA TAAAGGACTT 240

CCATTCACTT GCAAGGCTTT TGTTTTTGAT AAAGCAAGAA AACAATGCCT CTGGTTCCCC 300

TTCAATAGCA TGTCAAGTGG AGTGAAAAAA GAATTTGGCC ATGAATTTGA CCTCTATGAA 360

AACAAAGACT ACATTAGAAA CTGCATCATT GGTAAAGGAC GCAGCTACAA GGGAACAGTA 420

TCTATCACTA AGAGTGGCAT CAAATGTCAG CCCTGGAGTT CCATGATACC ACACGAACAC 480

AGCTATCGGG GTAAAGACCT ACAGGAAAAC TACTGTCGAA ATCCTCGAGG GGAAGAAGGG 540

GGACCCTGGT GTTTCACAAG CAATCCAGAG GTACGCTACG AAGTCTGTGA CATTCCTCAG 600

TGTTCAGAAG TTGAATGCAT GACCTGCAAT GGGGAGAGTT ATCGAGGTCT CATGGATCAT 660

ACAGAATCAG GCAAGATTTG TCAGCGCTGG GATCATCAGA CACCACACCG GCACAAATTC 720

TTGCCTGAAA GATATCCCGA CAAGGGCTTT GATGATAATT ATTGCCGCAA TCCCGATGGC 780

CAGCCGAGGC CATGGTGCTA TACTCTTGAC CCTCACACCC GCTGGGAGTA CTGTGCAATT 840

AAAACATGCG CTGACAAAGC TTCGGGCGGT GGCGGTTCTG GTGGCGGTGG CTCCGGCGGT 900

GGCGGTTCTC TAGAGGGACA AAGGAAAAGA AGAAATACAA TTCATGAATT CAAAAAATCA 960

GCAAAGACTA CCCTAATCAA AATAGATCCA GCACTGAAGA TAAAAACCAA AAAAGTGAAT 1020

ACTGCAGACC AATGTGCTAA TAGATGTACT AGGAATAAAG GACTTCCATT CACTTGCAAG 1080

GCTTTTGTTT TTGATAAAGC AAGAAAACAA TGCCTCTGGT TCCCCTTCAA TAGCATGTCA 1140

AGTGGAGTGA AAAAAGAATT TGGCCATGAA TTTGACCTCT ATGAAAACAA AGACTACATT 1200

AGAAACTGCA TCATTGGTAA AGGACGCAGC TACAAGGGAA CAGTATCTAT CACTAAGAGT 1260

GGCATCAAAT GTCAGCCCTG GAGTTCCATG ATACCACACG AACACAGCTA TCGGGGTAAA 1320

GACCTACAGG AAAACTACTG TCGAAATCCT CGAGGGGAAG AAGGGGGACC CTGGTGTTTC 1380

ACAAGCAATC CAGAGGTACG CTACGAAGTC TGTGACATTC CTCAGTGTTC AGAAGTTGAA 1440

TGCATGACCT GCAATGGGGA GAGTTATCGA GGTCTCATGG ATCATACAGA ATCAGGCAAG 1500

ATTTGTCAGC GCTGGGATCA TCAGACACCA CACCGGCACA AATTCTTGCC TGAAAGATAT 1560

CCCGACAAGG GCTTTGATGA TAATTATTGC CGCAATCCCG ATGGCCAGCC GAGGCCATGG 1620

TGCTATACTC TTGACCCTCA CACCCGCTGG GAGTACTGTG CAATTAAAAC ATGCGCTGAC 1680

AAAGCTGACG ACGACGACAA ACACCACCAC CACCACCACC ACTAG 1725

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 574 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Trp Val Thr Lys Leu Leu Pro Ala Leu Leu Leu Gln His Val Leu
1 5 10 15

Leu His Leu Leu Leu Leu Pro Ile Ala Ile Pro Tyr Ala Glu Gly Gln
20 25 30

Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser Ala Lys Thr
35 40 45

Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile Lys Thr Lys Lys Val
50 55 60

Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr Arg Asn Lys Gly Leu
65 70 75 80

Pro Phe Thr Cys Lys Ala Phe Val Phe Asp Lys Ala Arg Lys Gln Cys
85 90 95

Leu Trp Phe Pro Phe Asn Ser Met Ser Ser Gly Val Lys Lys Glu Phe
100 105 110

Gly His Glu Phe Asp Leu Tyr Glu Asn Lys Asp Tyr Ile Arg Asn Cys
115 120 125

Ile Ile Gly Lys Gly Arg Ser Tyr Lys Gly Thr Val Ser Ile Thr Lys
130 135 140

Ser Gly Ile Lys Cys Gln Pro Trp Ser Ser Met Ile Pro His Glu His
145 150 155 160

Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr Cys Arg Asn Pro Arg
165 170 175

Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser Asn Pro Glu Val Arg
180 185 190

Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu Val Glu Cys Met Thr
195 200 205

Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp His Thr Glu Ser Gly
210 215 220

Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro His Arg His Lys Phe
225 230 235 240

Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe Asp Asp Asn Tyr Cys Arg
245 250 255

Asn Pro Asp Gly Gln Pro Arg Pro Trp Cys Tyr Thr Leu Asp Pro His
260 265 270

Thr Arg Trp Glu Tyr Cys Ala Ile Lys Thr Cys Ala Asp Lys Ala Ser
275 280 285

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu
290 295 300

Glu Gly Gln Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser
305 310 315 320

Ala Lys Thr Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile Lys Thr
325 330 335

Lys Lys Val Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr Arg Asn
340 345 350

Lys Gly Leu Pro Phe Thr Cys Lys Ala Phe Val Phe Asp Lys Ala Arg
355 360 365

Lys Gln Cys Leu Trp Phe Pro Phe Asn Ser Met Ser Ser Gly Val Lys
370 375 380

Lys Glu Phe Gly His Glu Phe Asp Leu Tyr Glu Asn Lys Asp Tyr Ile
385 390 395 400

Arg Asn Cys Ile Ile Gly Lys Gly Arg Ser Tyr Lys Gly Thr Val Ser
405 410 415

Ile Thr Lys Ser Gly Ile Lys Cys Gln Pro Trp Ser Ser Met Ile Pro
420 425 430

His Glu His Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr Cys Arg
435 440 445

Asn Pro Arg Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser Asn Pro
450 455 460

Glu Val Arg Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu Val Glu
465 470 475 480

Cys Met Thr Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp His Thr
485 490 495

Glu Ser Gly Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro His Arg
500 505 510

His Lys Phe Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe Asp Asp Asn
515 520 525

Tyr Cys Arg Asn Pro Asp Gly Gln Pro Arg Pro Trp Cys Tyr Thr Leu
530 535 540

Asp Pro His Thr Arg Trp Glu Tyr Cys Ala Ile Lys Thr Cys Ala Asp
545 550 555 560

Lys Ala Asp Asp Asp Asp Lys His His His His His His His
565 570

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1692 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGGGGTGGC TCCCACTCCT GCTGCTTCTG ACTCAATGCT TAGGGGTCCC TGGGCAGCGC 60

TCGCCATTGA ATGACTTCCA AGTGCTCCGG GGCACAGAGC TACAGCACCT GCTACATGCG 120

GTGGTGCCCG GGCCTTGGCA GGAGGATGTG GCAGATGCTG AAGAGTGTGC TGGTCGCTGT 180

GGGCCCTTAA TGGACTGCCG GGCCTTCCAC TACAACGTGA GCAGCCATGG TTGCCAACTG 240

CTGCCATGGA CTCAACACTC GCCCCACACG AGGCTGCGGC GTTCTGGGCG CTGTGACCTC 300

TTCCAGAAGA AAGACTACGT ACGGACCTGC ATCATGAACA ATGGGGTTGG GTACCGGGGC 360

ACCATGGCCA CGACCGTGGG TGGCCTGCCC TGCCAGGCTT GGAGCCACAA GTTCCCGAAT 420

GATCACAAGT ACACGCCCAC TCTCCGGAAT GGCCTGGAAG AGAACTTCTG CCGTAACCCT 480

GATGGCGACC CCGGAGGTCC TTGGTGCTAC ACAACAGACC CTGCTGTGCG CTTCCAGAGC 540

TGCGGCATCA AATCCTGCCG GGAGGCCGCG TGTGTCTGGT GCAATGGCGA GGAATACCGC 600

GGCGCGGTAG ACCGCACGGA GTCAGGGCGC GAGTGCCAGC GCTGGGATCT TCAGCACCCG 660

CACCAGCACC CCTTCGAGCC GGGCAAGTTC CTCGACCAAG GTCTGGACGA CAACTATTGC 720

CGGAATCCTG ACGGCTCCGA GCGGCCATGG TGCTACACTA CGGATCCGCA GATCGAGCGA 780

GAGTTCTGTG ACCTCCCCCG CTGCGGGTCC GAGGCACAGC CCCGCCTCGA GGGCGGTGGC 840

GGTTCTGGTG GCGGTGGCTC CGGCGGTGGC GGTTCTCTAG AGGGACAAAG GAAAAGAAGA 900

AATACAATTC ATGAATTCAA AAAATCAGCA AAGACTACCC TAATCAAAAT AGATCCAGCA 960

CTGAAGATAA AAACCAAAAA AGTGAATACT GCAGACCAAT GTGCTAATAG ATGTACTAGG 1020

AATAAAGGAC TTCCATTCAC TTGCAAGGCT TTTGTTTTTG ATAAAGCAAG AAAACAATGC 1080

CTCTGGTTCC CCTTCAATAG CATGTCAAGT GGAGTGAAAA AAGAATTTGG CCATGAATTT 1140

GACCTCTATG AAAACAAAGA CTACATTAGA AACTGCATCA TTGGTAAAGG ACGCAGCTAC 1200

AAGGGAACAG TATCTATCAC TAAGAGTGGC ATCAAATGTC AGCCCTGGAG TTCCATGATA 1260

CCACACGAAC ACAGCTATCG GGGTAAAGAC CTACAGGAAA ACTACTGTCG AAATCCTCGA 1320

GGGGAAGAAG GGGGACCCTG GTGTTTCACA AGCAATCCAG AGGTACGCTA CGAAGTCTGT 1380

GACATTCCTC AGTGTTCAGA AGTTGAATGC ATGACCTGCA ATGGGGAGAG TTATCGAGGT 1440

CTCATGGATC ATACAGAATC AGGCAAGATT TGTCAGCGCT GGGATCATCA GACACCACAC 1500

CGGCACAAAT TCTTGCCTGA AAGATATCCC GACAAGGGCT TTGATGATAA TTATTGCCGC 1560

AATCCCGATG GCCAGCCGAG GCCATGGTGC TATACTCTTG ACCCTCACAC CCGCTGGGAG 1620

TACTGTGCAA TTAAAACATG CGCTGACAAA GCTGACGACG ACGACAAACA CCACCACCAC 1680

CACCACCACT AG 1692

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 563 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Trp Leu Pro Leu Leu Leu Leu Thr Gln Cys Leu Gly Val
1 5 10 15

Pro Gly Gln Arg Ser Pro Leu Asn Asp Phe Gln Val Leu Arg Gly Thr
20 25 30

Glu Leu Gln His Leu Leu His Ala Val Val Pro Gly Pro Trp Gln Glu
35 40 45

Asp Val Ala Asp Ala Glu Glu Cys Ala Gly Arg Cys Gly Pro Leu Met
50 55 60

Asp Cys Arg Ala Phe His Tyr Asn Val Ser Ser His Gly Cys Gln Leu
65 70 75 80

Leu Pro Trp Thr Gln His Ser Pro His Thr Arg Leu Arg Arg Ser Gly
85 90 95

Arg Cys Asp Leu Phe Gln Lys Lys Asp Tyr Val Arg Thr Cys Ile Met
100 105 110

Asn Asn Gly Val Gly Tyr Arg Gly Thr Met Ala Thr Thr Val Gly Gly
115 120 125

Leu Pro Cys Gln Ala Trp Ser His Lys Phe Pro Asn Asp His Lys Tyr
130 135 140

Thr Pro Thr Leu Arg Asn Gly Leu Glu Glu Asn Phe Cys Arg Asn Pro
145 150 155 160

Asp Gly Asp Pro Gly Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ala Val
165 170 175

Arg Phe Gln Ser Cys Gly Ile Lys Ser Cys Arg Glu Ala Ala Cys Val
180 185 190

Trp Cys Asn Gly Glu Glu Tyr Arg Gly Ala Val Asp Arg Thr Glu Ser
195 200 205

Gly Arg Glu Cys Gln Arg Trp Asp Leu Gln His Pro His Gln His Pro
210 215 220

Phe Glu Pro Gly Lys Phe Leu Asp Gln Gly Leu Asp Asp Asn Tyr Cys
225 230 235 240

Arg Asn Pro Asp Gly Ser Glu Arg Pro Trp Cys Tyr Thr Thr Asp Pro
245 250 255

Gln Ile Glu Arg Glu Phe Cys Asp Leu Pro Arg Cys Gly Ser Glu Ala
260 265 270

Gln Pro Arg Leu Glu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
275 280 285

Gly Gly Gly Ser Leu Glu Gly Gln Arg Lys Arg Arg Asn Thr Ile His
290 295 300

Glu Phe Lys Lys Ser Ala Lys Thr Thr Leu Ile Lys Ile Asp Pro Ala
305 310 315 320

Leu Lys Ile Lys Thr Lys Lys Val Asn Thr Ala Asp Gln Cys Ala Asn
325 330 335

Arg Cys Thr Arg Asn Lys Gly Leu Pro Phe Thr Cys Lys Ala Phe Val
340 345 350

Phe Asp Lys Ala Arg Lys Gln Cys Leu Trp Phe Pro Phe Asn Ser Met
355 360 365

Ser Ser Gly Val Lys Lys Glu Phe Gly His Glu Phe Asp Leu Tyr Glu
370 375 380

Asn Lys Asp Tyr Ile Arg Asn Cys Ile Ile Gly Lys Gly Arg Ser Tyr
385 390 395 400

Lys Gly Thr Val Ser Ile Thr Lys Ser Gly Ile Lys Cys Gln Pro Trp
405 410 415

Ser Ser Met Ile Pro His Glu His Ser Tyr Arg Gly Lys Asp Leu Gln
420 425 430

Glu Asn Tyr Cys Arg Asn Pro Arg Gly Glu Glu Gly Gly Pro Trp Cys
435 440 445

Phe Thr Ser Asn Pro Glu Val Arg Tyr Glu Val Cys Asp Ile Pro Gln
450 455 460

Cys Ser Glu Val Glu Cys Met Thr Cys Asn Gly Glu Ser Tyr Arg Gly
465 470 475 480

Leu Met Asp His Thr Glu Ser Gly Lys Ile Cys Gln Arg Trp Asp His
485 490 495

Gln Thr Pro His Arg His Lys Phe Leu Pro Glu Arg Tyr Pro Asp Lys
500 505 510

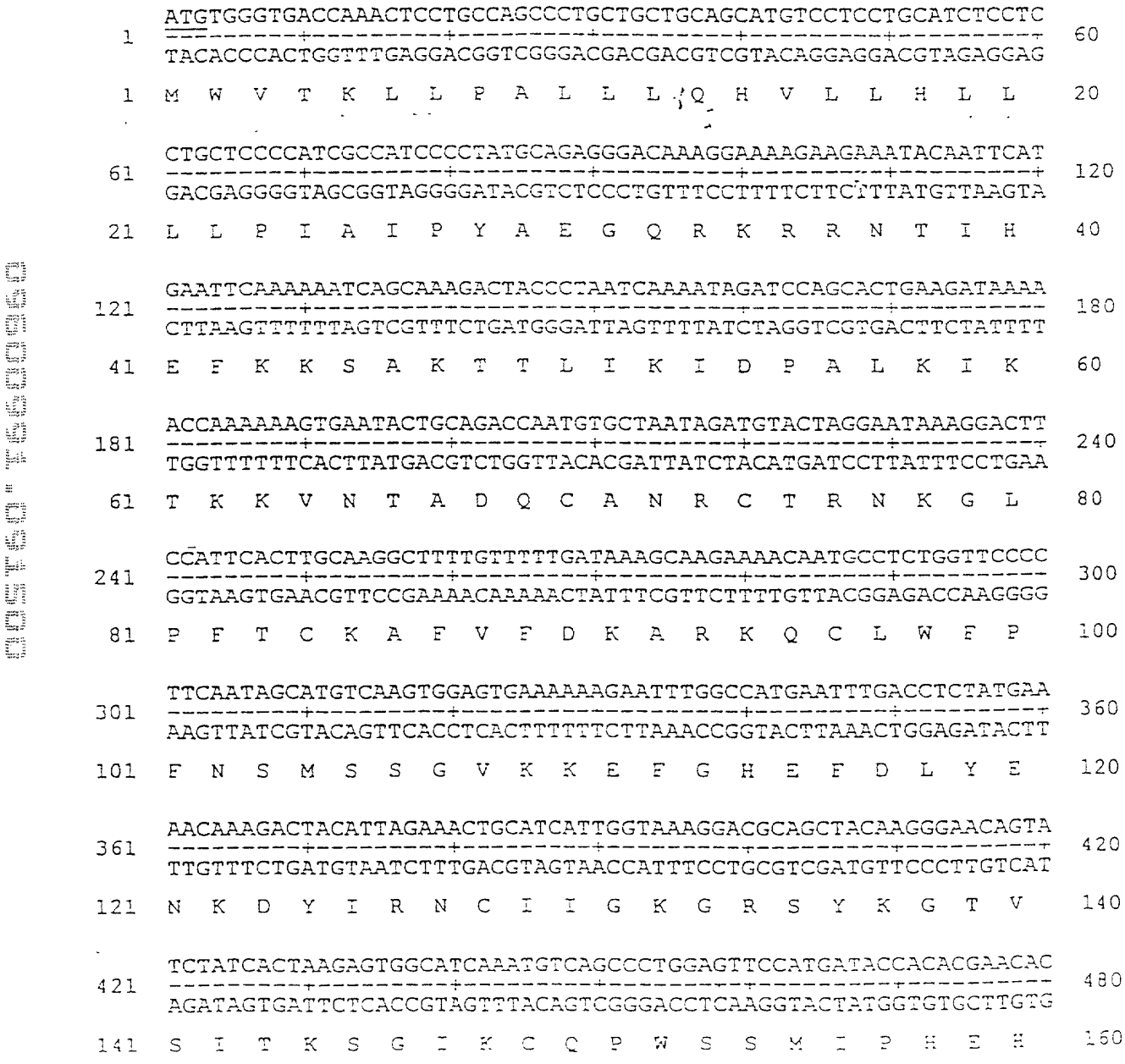
Gly Phe Asp Asp Asn Tyr Cys Arg Asn Pro Asp Gly Gln Pro Arg Pro
515 520 525

Trp Cys Tyr Thr Leu Asp Pro His Thr Arg Trp Glu Tyr Cys Ala Ile
530 535 540

Lys Thr Cys Ala Asp Lys Ala Asp Asp Asp Asp Lys His His His His
545 550 555 560

His His His

FIG 1a



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481  AGCTATCGGGGTAAAGACCTACAGGAAAACTACTGTCGAAATCCTCGAGGGGAAGAAGGG  540
     ---------+---------+---------+---------+---------+---------+
     TCGATAGCCCCATTTCTGGATGTCCTTTTGATGACAGCTTTAGGAGCTCCCCTTCTTCCC

161  S  Y  R  G  K  D  L  Q  E  N  Y  C  R  N  P  R  G  E  E  G   180

541  GGACCCTGGTGTTTCACAAGCAATCCAGAGGTACGCTACGAAGTCTGTGACATTCCTCAG  600
     ---------+---------+---------+---------+---------+---------+
     CCTGGGACCACAAAGTGTTCGTTAGGTCTCCATGCGATGCTTCAGACACTGTAAGGAGTC

181  G  P  W  C  F  T  S  N  P  E  V  R  Y  E  V  C  D  I  P  Q   200

601  TGTTCAGAAGTTGAATGCATGACCTGCAATGGGGAGAGTTATCGAGGTCTCATGGATCAT  660
     ---------+---------+---------+---------+---------+---------+
     ACAAGTCTTCAACTTACGTACTGGACGTTACCCCTCTCAATAGCTCCAGAGTACCTAGTA

201  C  S  E  V  E  C  M  T  C  N  G  E  S  Y  R  G  L  M  D  H   220

661  ACAGAATCAGGCAAGATTTGTCAGCGCTGGGATCATCAGACACCACACCGGCACAAATTC  720
     ---------+---------+---------+---------+---------+---------+
     TGTCTTAGTCCGTTCTAAACAGTCGCGACCCTAGTAGTCTGTGGTGTGGCCGTGTTTAAG

221  T  E  S  G  K  I  C  Q  R  W  D  H  Q  T  P  H  R  H  K  F   240

721  TTGCCTGAAAGATATCCCGACAAGGGCTTTGATGATAATTATTGCCGCAATCCCGATGGC  780
     ---------+---------+---------+---------+---------+---------+
     AACGGACTTTCTATAGGGCTGTTCCCGAAACTACTATTAATAACGGCGTTAGGGCTACCG

241  L  P  E  R  Y  P  D  K  G  F  D  D  N  Y  C  R  N  P  D  G   260

781  CAGCCGAGGCCATGGTGCTATACTCTTGACCCTCACACCCGCTGGGAGTACTGTGCAATT  840
     ---------+---------+---------+---------+---------+---------+
     GTCGGCTCCGGTACCACGATATGAGAACTGGGAGTGTGGGCGACCCTCATGACACGTTAA

261  Q  P  R  P  W  C  Y  T  L  D  P  H  T  R  W  E  Y  C  A  I   280

841  AAAACATGCGCTGACAATACTATGAATGACACTGATGTTCCTTTGGAAACAACTGAATGC  900
     ---------+---------+---------+---------+---------+---------+
     TTTTGTACGCGACTGTTATGATACTTACTGTGACTACAAGGAAACCTTTGTTGACTTACG

281  K  T  C  A  D  N  T  M  N  D  T  D  V  P  L  E  T  T  E  C   300

901  ATCCAAGGTCAAGGAGAAGGCTACAGGGGCACTGTCAATACCATTTGGAATGGAATTCCA  960
     ---------+---------+---------+---------+---------+---------+
     TAGGTTCCAGTTCCTCTTCCGATGTCCCCGTGACAGTTATGGTAAACCTTACCTTAAGGT

301  I  Q  G  Q  G  E  G  Y  R  G  T  V  N  T  I  W  N  G  I  P   320

961  TGTCAGCGTTGGGATTCTCAGTATCCTCACGAGCATGACATGACTCCTGAAAATTTCAAG  1020
     ---------+---------+---------+---------+---------+---------+
     ACAGTCGCAACCCTAAGAGTCATAGGAGTGCTCGTACTGTACTGAGGACTTTTAAAGTTC

321  C  Q  R  W  D  S  Q  Y  P  H  E  H  D  M  T  P  E  N  F  K   340

1021 TGCAAGGACCTACGAGAAAATTACTGCCGAAATCCAGATGGGTCTGAATCACCCTGGTGT  1080
     ---------+---------+---------+---------+---------+---------+
     ACGTTCCTGGATGCTCTTTTAATGACGGCTTTAGGTCTACCCAGACTTAGTGGGACCACA

341  C  K  D  L  R  E  N  Y  C  R  N  P  D  G  S  E  S  P  W  C   360

1081 TTTACCACTGATCCAAACATCCGAGTTGGCTACTGCTCCCAAATTCCAAACTGTGATATG  1140
     ---------+---------+---------+---------+---------+---------+
     AAATGGTGACTAGGTTTGTAGGCTCAACCGATGACGAGGGTTTAAGGTTTGACACTATAC

361  F  T  T  D  P  N  I  R  V  G  Y  C  S  Q  I  P  N  C  D  M   380
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1141 TCACATGGACAAGATTGTTATCGTGGGAATGGCAAAAATTATATGGGCAACTTATCCCAA 1200
     ---------+---------+---------+---------+---------+---------+
     AGTGTACCTGTTCTAACAATAGCACCCTTACCGTTTTTAATATACCCGTTGAATAGGGTT

 381 S  H  G  Q  D  C  Y  R  G  N  G  K  N  Y  M  G  N  L  S  Q   400

1201 ACAAGATCTGGACTAACATGTTCAATGTGGGACAAGAACATGGAAGACTTACATCGTCAT 1260
     ---------+---------+---------+---------+---------+---------+
     TGTTCTAGACCTGATTGTACAAGTTACACCCTGTTCTTGTACCTTCTGAATGTAGCAGTA

 401 T  R  S  G  L  T  C  S  M  W  D  K  N  M  E  D  L  H  R  H   420

1261 ATCTTCTGGGAACCAGATGCAAGTAAGCTGAATGAGAATTACTGCCGAAATCCAGATGAT 1320
     ---------+---------+---------+---------+---------+---------+
     TAGAAGACCCTTGGTCTACGTTCATTCGACTTACTCTTAATGACGGCTTTAGGTCTACTA

 421 I  F  W  E  P  D  A  S  K  L  N  E  N  Y  C  R  N  P  D  D   440

1321 GACGCTCATGGACCCTGGTGCTACACGGGAAATCCACTCATTCCTTGGGATTATTGCCCT 1380
     ---------+---------+---------+---------+---------+---------+
     CTGCGAGTACCTGGGACCACGATGTGCCCTTTAGGTGAGTAAGGAACCCTAATAACGGGA

 441 D  A  H  G  P  W  C  Y  T  G  N  P  L  I  P  W  D  Y  C  P   460

1381 ATTTCTCGTTGTGAAGGTGATACCACACCTACAATAGTCAATTTAGACCATCCCGTAATA 1440
     ---------+---------+---------+---------+---------+---------+
     TAAAGAGCAACACTTCCACTATGGTGTGGATGTTATCAGTTAAATCTGGTAGGGCATTAT

 461 I  S  R  C  E  G  D  T  T  P  T  I  V  N  L  D  H  P  V  I   480

1441 TCTTGTGCCAAAACGAAACAATTGCGAGTTGTAAATGGGATTCCAACACGAACAAACATA 1500
     ---------+---------+---------+---------+---------+---------+
     AGAACACGGTTTTGCTTTGTTAACGCTCAACATTTACCCTAAGGTTGTGCTTGTTTGTAT

 481 S  C  A  K  T  K  Q  L  R  V  V  N  G  I  P  T  R  T  N  I   500

1501 GGATGGATGGTTAGTTTGAGATACAGAAATAAACATATCTGCGGAGGATCATTGATAAAG 1560
     ---------+---------+---------+---------+---------+---------+
     CCTACCTACCAATCAAACTCTATGTCTTTATTTGTATAGACGCCTCCTAGTAACTATTTC

 501 G  W  M  V  S  L  R  Y  R  N  K  H  I  C  G  G  S  L  I  K   520

1561 GAGAGTTGGGTTCTTACTGCACGACAGTGTTTCCCTTCTCGAGACTTGAAAGATTATGAA 1620
     ---------+---------+---------+---------+---------+---------+
     CTCTCAACCCAAGAATGACGTGCTGTCACAAAGGGAAGAGCTCTGAACTTTCTAATACTT

 521 E  S  W  V  L  T  A  R  Q  C  F  P  S  R  D  L  K  D  Y  E   540

1621 GCTTGGCTTGGAATTCATGATGTCCACGGAAGAGGAGATGAGAAATGCAAACAGGTTCTC 1680
     ---------+---------+---------+---------+---------+---------+
     CGAACCGAACCTTAAGTACTACAGGTGCCTTCTCCTCTACTCTTTACGTTTGTCCAAGAG

 541 A  W  L  G  I  H  D  V  H  G  R  G  D  E  K  C  K  Q  V  L   560

1681 AATGTTTCCCAGCTGGTATATGGCCCTGAAGGATCAGATCTGGTTTTAATGAAGCTTGCC 1740
     ---------+---------+---------+---------+---------+---------+
     TTACAAAGGGTCGACCATATACCGGGACTTCCTAGTCTAGACCAAAATTACTTCGAACGG

 561 N  V  S  Q  L  V  Y  G  P  E  G  S  D  L  V  L  M  K  L  A   580

1741 AGGCCTGCTGTCCTGGATGATTTTGTTAGTACGATTGATTTACCTAATTATGGATGCACA 1800
     ---------+---------+---------+---------+---------+---------+
     TCCGGACGACAGGACCTACTAAAACAATCATGCTAACTAAATGGATTAATACCTACGTGT

 581 R  P  A  V  L  D  D  F  V  S  T  I  D  L  P  N  Y  G  C  T   600
```

(continued)

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```
1801  ATTCCTGAAAAGACCAGTTGCAGTGTTTATGGCTGGGGCTACACTGGATTGATCAACTAT  1860
      ---------+---------+---------+---------+---------+---------+
      TAAGGACTTTTCTGGTCAACGTCACAAATACCGACCCCGATGTGACCTAACTAGTTGATA

 601  I  P  E  K  T  S  C  S  V  Y  G  W  G  Y  T  G  L  I  N  Y   620

1861  GATGGCCTATTACGAGTGGCACATCTCTATATAATGGGAAATGAGAAATGCAGCCAGCAT  1920
      ---------+---------+---------+---------+---------+---------+
      CTACCGGATAATGCTCACCGTGTAGAGATATATTACCCTTTACTCTTTACGTCGGTCGTA

 621  D  G  L  L  R  V  A  H  L  Y  I  M  G  N  E  K  C  S  Q  H   640

1921  CATCGAGGGAAGGTGACTCTGAATGAGTCTGAAATATGTGCTGGGGCTGAAAAGATTGGA  1980
      ---------+---------+---------+---------+---------+---------+
      GTAGCTCCCTTCCACTGAGACTTACTCAGACTTTATACACGACCCCGACTTTTCTAACCT

 641  H  R  G  K  V  T  L  N  E  S  E  I  C  A  G  A  E  K  I  G   660

1981  TCAGGACCATGTGAGGGGGATTATGGTGGCCCACTTGTTTGTGAGCAACATAAAATGAGA  2040
      ---------+---------+---------+---------+---------+---------+
      AGTCCTGGTACACTCCCCCTAATACCACCGGGTGAACAAACACTCGTTGTATTTTACTCT

 661  S  G  P  C  E  G  D  Y  G  G  P  L  V  C  E  Q  H  K  M  R   680

2041  ATGGTTCTTGGTGTCATTGTTCCTGGTCGTGGATGTGCCATTCCAAATCGTCCTGGTATT  2100
      ---------+---------+---------+---------+---------+---------+
      TACCAAGAACCACAGTAACAAGGACCAGCACCTACACGGTAAGGTTTAGCAGGACCATAA

 681  M  V  L  G  V  I  V  P  G  R  G  C  A  I  P  N  R  P  G  I   700

2101  TTTGTCCGAGTAGCATATTATGCAAAATGGATACACAAAATTATTTTAACATATAAGGTA  2160
      ---------+---------+---------+---------+---------+---------+
      AAACAGGCTCATCGTATAATACGTTTTACCTATGTGTTTTAATAAAATTGTATATTCCAT

 701  F  V  R  V  A  Y  Y  A  K  W  I  H  K  I  I  L  T  Y  K  V   720

2161  CCACAGTCATAG  2172
      ----------+==
      GGTGTCAGTATC

 721  P  Q  S  *    723
```

FIG 1b

```
1   ATGGGGTGGCTCCCACTCCTGCTGCTTCTGACTCAATGCTTAGGGGTCCCTGGGCAGCGC   60
    TACCCCACCGAGGGTGAGGACGACGAAGACTGAGTTACGAATCCCCAGGGACCCGTCGCG

1   M  G  W  L  P  L  L  L  L  T  Q  C  L  G  V  P  G  Q  R      20

61  TCGCCATTGAATGACTTCCAAGTGCTCCGGGGCACAGAGCTACAGCACCTGCTACATGCG   120
    AGCGGTAACTTACTGAAGGTTCACGAGGCCCCGTGTCTCGATGTCGTGGACGATGTACGC

21  S  P  L  N  D  F  Q  V  L  R  G  T  E  L  Q  H  L  L  H  A    40

121 GTGGTGCCCGGGCCTTGGCAGGAGGATGTGGCAGATGCTGAAGAGTGTGCTGGTCGCTGT   180
    CACCACGGGCCCGGAACCGTCCTCCTACACCGTCTACGACTTCTCACACGACCAGCGACA

41  V  V  P  G  P  W  Q  E  D  V  A  D  A  E  E  C  A  G  R  C    60

181 GGGCCCTTAATGGACTGCCGGGCCTTCCACTACAACGTGAGCAGCCATGGTTGCCAACTG   240
    CCCGGGAATTACCTGACGGCCCGGAAGGTGATGTTGCACTCGTCGGTACCAACGGTTGAC

61  G  P  L  M  D  C  R  A  F  H  Y  N  V  S  S  H  G  C  Q  L    80

241 CTGCCATGGACTCAACACTCGCCCCACACGAGGCTGCGGCGTTCTGGGCGCTGTGACCTC   300
    GACGGTACCTGAGTTGTGAGCGGGGTGTGCTCCGACGCCGCAAGACCCGCGACACTGGAG

81  L  P  W  T  Q  H  S  P  H  T  R  L  R  R  S  G  R  C  D  L   100

301 TTCCAGAAGAAAGACTACGTACGGACCTGCATCATGAACAATGGGGTTGGGTACCGGGGC   360
    AAGGTCTTCTTTCTGATGCATGCCTGGACGTAGTACTTGTTACCCCAACCCATGGCCCCG

101 F  Q  K  K  D  Y  V  R  T  C  I  M  N  N  G  V  G  Y  R  G   120

361 ACCATGGCCACGACCGTGGGTGGCCTGCCCTGCCAGGCTTGGAGCCACAAGTTCCCGAAT   420
    TGGTACCGGTGCTGGCACCCACCGGACGGGACGGTCCGAACCTCGGTGTTCAAGGGCTTA

121 T  M  A  T  T  V  G  G  L  P  C  Q  A  W  S  H  K  F  P  N   140

421 GATCACAAGTACACGCCCACTCTCCGGAATGGCCTGGAAGAGAACTTCTGCCGTAACCCT   480
    CTAGTGTTCATGTGCGGGTGAGAGGCCTTACCGGACCTTCTCTTGAAGACGGCATTGGGA

141 D  H  K  Y  T  P  T  L  R  N  G  L  E  E  N  F  C  R  N  P   160
```

(continued)

(continued)

```
481  GATGGCGACCCCGGAGGTCCTTGGTGCTACACAACAGACCCTGCTGTGCGCTTCCAGAGC
     ---------+---------+---------+---------+---------+---------+---------- 540
     CTACCGCTGGGGCCTCCAGGAACCACGATGTGTTGTCTGGGACGACACGCGAAGGTCTCG

161  D  G  D  P  G  G  P  W  C  Y  T  T  D  P  A  V  R  F  Q  S    180

531  TGCGGCATCAAATCCTGCCGGGAGGCCGCGTGTGTCTGGTGCAATGGCGAGGAATACCGC
     ---------+---------+---------+---------+---------+---------+---------- 600
     ACGCCGTAGTTTAGGACGGCCCTCCGGCGCACACAGACCACGTTACCGCTCCTTATGGCG

181  C  G  I  K  S  C  R  E  A  A  C  V  W  C  N  G  E  E  Y  R    200

601  GGCGCGGTAGACCGCACGGAGTCAGGGCGCGAGTGCCAGCGCTGGGATCTTCAGCACCCG
     ---------+---------+---------+---------+---------+---------+---------- 660
     CCGCGCCATCTGGCGTGCCTCAGTCCCGCGCTCACGGTCGCGACCCTAGAAGTCGTGGGC

201  G  A  V  D  R  T  E  S  G  R  E  C  Q  R  W  D  L  Q  H  P    220

661  CACCAGCACCCCTTCGAGCCGGGCAAGTTCCTCGACCAAGGTCTGGACGACAACTATTGC
     ---------+---------+---------+---------+---------+---------+---------- 720
     GTGGTCGTGGGGAAGCTCGGCCCGTTCAAGGAGCTGGTTCCAGACCTGCTGTTGATAACG

221  H  Q  H  P  F  E  P  G  K  F  L  D  Q  G  L  D  D  N  Y  C    240

721  CGGAATCCTGACGGCTCCGAGCGGCCATGGTGCTACACTACGGATCCGCAGATCGAGCGA
     ---------+---------+---------+---------+---------+---------+---------- 780
     GCCTTAGGACTGCCGAGGCTCGCCGGTACCACGATGTGATGCCTAGGCGTCTAGCTCGCT

241  R  N  P  D  G  S  E  R  P  W  C  Y  T  T  D  P  Q  I  E  R    260

781  GAGTTCTGTGACCTCCCCCGCTGCGGGTCCGAGGCACAGCCCCGCCAAGAGGCCACAACT
     ---------+---------+---------+---------+---------+---------+---------- 840
     CTCAAGACACTGGAGGGGGCGACGCCCAGGCTCCGTGTCGGGGCGGTTCTCCGGTGTTGA

261  E  F  C  D  L  P  R  C  G  S  E  A  Q  P  R  Q  E  A  T  T    280

841  GTCAGCTGCTTCCGCGGGAAGGGTGAGGGCTACCGGGGCACAGCCAATACCACCACTGCG
     ---------+---------+---------+---------+---------+---------+---------- 900
     CAGTCGACGAAGGCGCCCTTCCCACTCCCGATGGCCCCGTGTCGGTTATGGTGGTGACGC

281  V  S  C  F  R  G  K  G  E  G  Y  R  G  T  A  N  T  T  T  A    300

901  GGCGTACCTTGCCAGCGTTGGGACGCGCAAATCCCGCATCAGCACCGATTTACGCCAGAA
     ---------+---------+---------+---------+---------+---------+---------- 960
     CCGCATGGAACGGTCGCAACCCTGCGCGTTTAGGGCGTAGTCGTGGCTAAATGCGGTCTT

301  G  V  P  C  Q  R  W  D  A  Q  I  P  H  Q  H  R  F  T  P  E    320

961  AAATACGCGTGCAAAGACCTTCGGGAGAACTTCTGCCGGAACCCCGACGGCTCAGAGGCG
     ---------+---------+---------+---------+---------+---------+---------- 1020
     TTTATGCGCACGTTTCTGGAAGCCCTCTTGAAGACGGCCTTGGGGCTGCCGAGTCTCCGC

321  K  Y  A  C  K  D  L  R  E  N  F  C  R  N  P  D  G  S  E  A    340

1021 CCCTGGTGCTTCACACTGCGGCCCGGCATGCGCGCGGCCTTTTGCTACCAGATCCGGCGT
     ---------+---------+---------+---------+---------+---------+---------- 1080
     GGGACCACGAAGTGTGACGCCGGGCCGTACGCGCGCCGGAAAACGATGGTCTAGGCCGCA

341  P  W  C  F  T  L  R  P  G  M  R  A  A  F  C  Y  Q  I  R  R    360

1081 TGTACAGACGACGTGCGGCCCCAGGACTGCTACCACGGCGCAGGGGAGCAGTACCGCGGC
     ---------+---------+---------+---------+---------+---------+---------- 1140
     ACATGTCTGCTGCACGCCGGGGTCCTGACGATGGTGCCGCGTCCCCTCGTCATGGCGCCG

361  C  T  D  D  V  R  P  Q  D  C  Y  H  G  A  G  E  Q  Y  R  G    380
```

(continued)

(continued)

```
                ACGGTCAGCAAGACCCGCAAGGGTGTCCAGTGCCAGCGCTGGTCCGCTGAGACGCCGCAC
1141            ----------+---------+---------+---------+---------+---------- 1200
                TGCCAGTCGTTCTGGGCGTTCCCACAGGTCACGGTCGCGACCAGGCGACTCTGCGGCGTG

 381  T  V  S  K  T  R  K  G  V  Q  C  Q  R  W  S  A  E  T  P  H    400

                AAGCCGCAGTTCACGTTTACCTCCGAACCGCATGCACAACTGGAGGAGAACTTCTGCCGG
1201            ----------+---------+---------+---------+---------+---------- 1260
                TTCGGCGTCAAGTGCAAATGGAGGCTTGGCGTACGTGTTGACCTCCTCTTGAAGACGGCC

 401  K  P  Q  F  T  F  T  S  E  P  H  A  Q  L  E  E  N  F  C  R    420

                AACCCAGATGGGGATAGCCATGGGCCCTGGTGCTACACGATGGACCCAAGGACCCCATTC
1261            ----------+---------+---------+---------+---------+---------- 1320
                TTGGGTCTACCCCTATCGGTACCCGGGACCACGATGTGCTACCTGGGTTCCTGGGGTAAG

 421  N  P  D  G  D  S  H  G  P  W  C  Y  T  M  D  P  R  T  P  F    440

                GACTACTGTGCCCTGCGACGCTGCGCTGATGACCAGCCGCCATCAATCCTGGACCCCCCA
1321            ----------+---------+---------+---------+---------+---------- 1380
                CTGATGACACGGGACGCTGCGACGCGACTACTGGTCGGCGGTAGTTAGGACCTGGGGGGT

 441  D  Y  C  A  L  R  R  C  A  D  D  Q  P  P  S  I  L  D  P  P    460

                GACCAGGTGCAGTTTGAGAAGTGTGGCAAGAGGGTGGATCGGCTGGATCAGCGGCGTTCC
1381            ----------+---------+---------+---------+---------+---------- 1440
                CTGGTCCACGTCAAACTCTTCACACCGTTCTCCCACCTAGCCGACCTAGTCGCCGCAAGG

 461  D  Q  V  Q  F  E  K  C  G  K  R  V  D  R  L  D  Q  R  R  S    480

                AAGCTGCGCGTGGTTGGGGGCCATCCGGGCAACTCACCCTGGACAGTCAGCTTGCGGAAT
1441            ----------+---------+---------+---------+---------+---------- 1500
                TTCGACGCGCACCAACCCCCGGTAGGCCCGTTGAGTGGGACCTGTCAGTCGAACGCCTTA

 481  K  L  R  V  V  G  G  H  P  G  N  S  P  W  T  V  S  L  R  N    500

                CGGCAGGGCCAGCATTTCTGCGGGGGGTCTCTAGTGAAGGAGCAGTGGATACTGACTGCC
1501            ----------+---------+---------+---------+---------+---------- 1560
                GCCGTCCCGGTCGTAAAGACGCCCCCCAGAGATCACTTCCTCGTCACCTATGACTGACGG

 501  R  Q  G  Q  H  F  C  G  G  S  L  V  K  E  Q  W  I  L  T  A    520

                CGGCAGTGCTTCTCCTCCTGCCATATGCCTCTCACGGGCTATGAGGTATGGTTGGGCACC
1561            ----------+---------+---------+---------+---------+---------- 1620
                GCCGTCACGAAGAGGAGGACGGTATACGGAGAGTGCCCGATACTCCATACCAACCCGTGG

 521  R  Q  C  F  S  S  C  H  M  P  L  T  G  Y  E  V  W  L  G  T    540

                CTGTTCCAGAACCCACAGCATGGAGAGCCAAGCCTACAGCGGGTCCCAGTAGCCAAGATG
1621            ----------+---------+---------+---------+---------+---------- 1680
                GACAAGGTCTTGGGTGTCGTACCTCTCGGTTCGGATGTCGCCCAGGGTCATCGGTTCTAC

 541  L  F  Q  N  P  Q  H  G  E  P  S  L  Q  R  V  P  V  A  K  M    560

                GTGTGTGGGCCCTCAGGCTCCCAGCTTGTCCTGCTCAAGCTGGAGAGATCTGTGACCCTG
1681            ----------+---------+---------+---------+---------+---------- 1740
                CACACACCCGGGAGTCCGAGGGTCGAACAGGACGAGTTCGACCTCTCTAGACACTGGGAC

 561  V  C  G  P  S  G  S  Q  L  V  L  L  K  L  E  R  S  V  T  L    580

                AACCAGCGTGTGGCCCTGATCTGCCTGCCCCCTGAATGGTATGTGGTGCCTCCAGGGACC
1741            ----------+---------+---------+---------+---------+---------- 1800
                TTGGTCGCACACCGGGACTAGACGGACGGGGGACTTACCATACACCACGGAGGTCCCTGG

 581  N  Q  R  V  A  L  I  C  L  P  P  E  W  Y  V  V  P  P  G  T    600
```

(continued)

(continued)

```
1801  AAGTGTGAGATTGCAGGCTGGGGTGAGACCAAAGGTACGGGTAATGACACAGTCCTAAAT  1860
      ---------+---------+---------+---------+---------+---------+
      TTCACACTCTAACGTCCGACCCCACTCTGGTTTCCATGCCCATTACTGTGTCAGGATTTA

 601  K  C  E  I  A  G  W  G  E  T  K  G  T  G  N  D  T  V  L  N   620

1861  GTGGCCTTTCTGAATGTTATCTCCAACCAGGAGTGTAACATCAAGCACCGAGGACGTGTG  1920
      ---------+---------+---------+---------+---------+---------+
      CACCGGAAAGACTTACAATAGAGGTTGGTCCTCACATTGTAGTTCGTGGCTCCTGCACAC

 621  V  A  F  L  N  V  I  S  N  Q  E  C  N  I  K  H  R  G  R  V   640

1921  CGGGAGAGTGAGATGTGCACTGAGGGACTGTTGGCCCCTGTGGGGGGCCTGTGAGGGTGAC  1980
      ---------+---------+---------+---------+---------+---------+
      GCCCTCTCACTCTACACGTGACTCCCTGACAACCGGGGACACCCCCGGACACTCCCACTG

 641  R  E  S  E  M  C  T  E  G  L  L  A  P  V  G  A  C  E  G  D   660

1981  TACGGGGGCCCACTTGCCTGCTTTACCCACAACTGCTGGGTCCTGGAAGGAATTATAATC  2040
      ---------+---------+---------+---------+---------+---------+
      ATGCCCCCGGGTGAACGGACGAAATGGGTGTTGACGACCCAGGACCTTCCTTAATATTAG

 661  Y  G  G  P  L  A  C  F  T  H  N  C  W  V  L  E  G  I  I  I   680

2041  CCCAACCGAGTATGCGCAAGGTCCCGCTGGCCAGCTGTCTTCACGCGTGTCTCTGTGTTT  2100
      ---------+---------+---------+---------+---------+---------+
      GGGTTGGCTCATACGCGTTCCAGGGCGACCGGTCGACAGAAGTGCGCACAGAGACACAAA

 681  P  N  R  V  C  A  R  S  R  W  P  A  V  F  T  R  V  S  V  F   700

2101  GTGGACTGGATTCACAAGGTCATGAGACTGGGTTAG                          2136
      ---------+---------+---------+-------
      CACCTGACCTAAGTGTTCCAGTACTCTGACCCAATC

 701  V  D  W  I  H  K  V  M  R  L  G  *                           711
```

FIG 2a

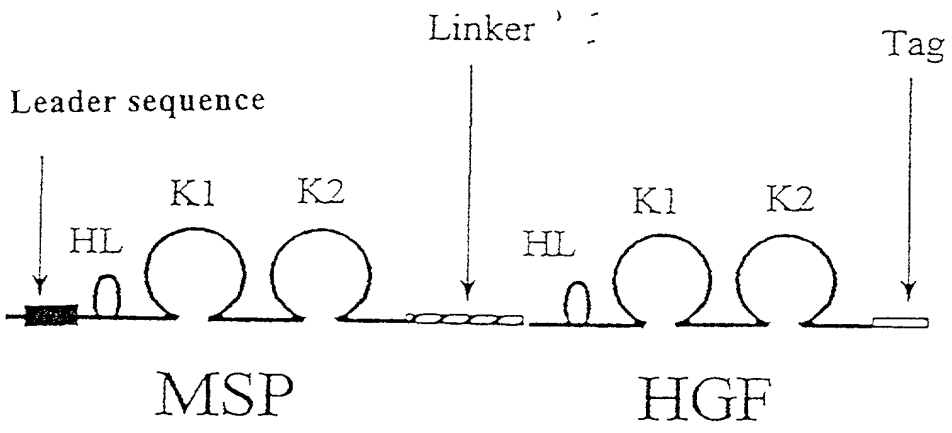

FIG 2b

```
1   GAATTCCACCATGGGGTGGCTCCCACTCCTGCTGCTTCTGACTCAATGCTTAGGGGTCCC   60
    CTTAAGGTGGTACCCCACCGAGGGTGAGGACGACGAAGACTGAGTTACGAATCCCCAGGG

1                M  G  W  L  P  L  L  L  L  L  T  Q  C  L  G  V  P   17

61  TGGGCAGCGCTCGCCATTGAATGACTTCCAAGTGCTCCGGGGCACAGAGCTACAGCACCT   120
    ACCCGTCGCGAGCGGTAACTTACTGAAGGTTCACGAGGCCCCGTGTCTCGATGTCGTGGA

18   G  Q  R  S  P  L  N  D  F  Q  V  L  R  G  T  E  L  Q  H  L   37

121 GCTACATGCGGTGGTGCCCGGGCCTTGGCAGGAGGATGTGGCAGATGCTGAAGAGTGTGC   180
    CGATGTACGCCACCACGGGCCCGGAACCGTCCTCCTACACCGTCTACGACTTCTCACACG

38   L  H  A  V  V  P  G  P  W  Q  E  D  V  A  D  A  E  E  C  A   57

181 TGGTCGCTGTGGGCCCTTAATGGACTGCCGGGCCTTCCACTACAACGTGAGCAGCCATGG   240
    ACCAGCGACACCCGGGAATTACCTGACGGCCCGGAAGGTGATGTTGCACTCGTCGGTACC

58   G  R  C  G  P  L  M  D  C  R  A  F  H  Y  N  V  S  S  H  G   77

241 TTGCCAACTGCTGCCATGGACTCAACACTCGCCCCACACGAGGCTGCGGCGTTCTGGGCG   300
    AACGGTTGACGACGGTACCTGAGTTGTGAGCGGGGTGTGCTCCGACGCCGCAAGACCCGC

78   C  Q  L  L  P  W  T  Q  H  S  P  H  T  R  L  R  R  S  G  R   97

301 CTGTGACCTCTTCCAGAAGAAAGACTACGTACGGACCTGCATCATGAACAATGGGGTTGG   360
    GACACTGGAGAAGGTCTTCTTTCTGATGCATGCCTGGACGTAGTACTTGTTACCCCAACC

98   C  D  L  F  Q  K  K  D  Y  V  R  T  C  I  M  N  N  G  V  G   117

361 GTACCGGGGCACCATGGCCACGACCGTGGGTGGCCTGCCCTGCCAGGCTTGGAGCCACAA   420
    CATGGCCCCGTGGTACCGGTGCTGGCACCCACCGGACGGGACGGTCCGAACCTCGGTGTT

118  Y  R  G  T  M  A  T  T  V  G  G  L  P  C  Q  A  W  S  H  K   137

421 GTTCCCGAATGATCACAAGTACACGCCCACTCTCCGGAATGGCCTGGAAGAGAACTTCTG   480
    CAAGGGCTTACTAGTGTTCATGTGCGGGTGAGAGGCCTTACCGGACCTTCTCTTGAAGAC

138  F  P  N  D  H  K  Y  T  P  T  L  R  N  G  L  E  E  N  F  C   157

481 CCGTAACCCTGATGGCGACCCCGGAGGTCCTTGGTGCTACACAACAGACCCTGCTGTGCG   540
    GGCATTGGGACTACCGCTGGGGCCTCCAGGAACCACGATGTGTTGTCTGGGACGACACGC

158  R  N  P  D  G  D  P  G  G  P  W  C  Y  T  T  D  P  A  V  R   177
```

(continued)

(continued)

```
541  CTTCCAGAGCTGCGGCATCAAATCCTGCCGGGAGGCCGCGTGTGTCTGGTGCAATGGCGA
     ----------+---------+---------+---------+---------+---------+---------- 600
     GAAGGTCTCGACGCCGTAGTTTAGGACGGCCCTCCGGCGCACACAGACCACGTTACCGCT

178  F  Q  S  C  G  I  K  S  C  R  E  A  A  C  V  W  C  N  G  E  197

601  GGAATACCGCGGCGCGGTAGACCGCACGGAGTCAGGGCGCGAGTGCCAGCGCTGGGATCT
     ----------+---------+---------+---------+---------+---------+---------- 660
     CCTTATGGCGCCGCGCCATCTGGCGTGCCTCAGTCCCGCGCTCACGGTCGCGACCCTAGA

198  E  Y  R  G  A  V  D  R  T  E  S  G  R  E  C  Q  R  W  D  L  217

661  TCAGCACCCGCACCAGCACCCCTTCGAGCCGGGCAAGTTCCTCGACCAAGGTCTGGACGA
     ----------+---------+---------+---------+---------+---------+---------- 720
     AGTCGTGGGCGTGGTCGTGGGGAAGCTCGGCCCGTTCAAGGAGCTGGTTCCAGACCTGCT

218  Q  H  P  H  Q  H  P  F  E  P  G  K  F  L  D  Q  G  L  D  D  237

721  CAACTATTGCCGGAATCCTGACGGCTCCGAGCGGCCATGGTGCTACACTACGGATCCGCA
     ----------+---------+---------+---------+---------+---------+---------- 780
     GTTGATAACGGCCTTAGGACTGCCGAGGCTCGCCGGTACCACGATGTGATGCCTAGGCGT

238  N  Y  C  R  N  P  D  G  S  E  R  P  W  C  Y  T  T  D  P  Q  257

781  GATCGAGCGAGAGTTCTGTGACCTCCCCCGCTGCGGGTCCGAGGCACAGCCCCGCCTCGA
     ----------+---------+---------+---------+---------+---------+---------- 840
     CTAGCTCGCTCTCAAGACACTGGAGGGGGCGACGCCCAGGCTCCGTGTCGGGGCGGAGCT

258  I  E  R  E  F  C  D  L  P  R  C  G  S  E  A  Q  P  R  L  E  277

841  GGGCGGTGGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTCTAGAGGGACAAAG
     ----------+---------+---------+---------+---------+---------+---------- 900
     CCCGCCACCGCCAAGACCACCGCCACCGAGGCCGCCACCGCCAAGAGATCTCCCTGTTTC

278  G  G  G  G  S  G  G  G  G  S  G  G  G  G  S  L  E  G  Q  R  297

901  GAAAAGAAGAAATACAATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAATCAAAAT
     ----------+---------+---------+---------+---------+---------+---------- 960
     CTTTTCTTCTTTATGTTAAGTACTTAAGTTTTTTAGTCGTTTCTGATGGGATTAGTTTTA

298  K  R  R  N  T  I  H  E  F  K  K  S  A  K  T  T  L  I  K  I  317

961  AGATCCAGCACTGAAGATAAAAACCAAAAAAGTGAATACTGCAGACCAATGTGCTAATAG
     ----------+---------+---------+---------+---------+---------+---------- 1020
     TCTAGGTCGTGACTTCTATTTTTGGTTTTTTCACTTATGACGTCTGGTTACACGATTATC

318  D  P  A  L  K  I  K  T  K  K  V  N  T  A  D  Q  C  A  N  R  337

1021 ATGTACTAGGAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTTTGATAAAGCAAG
     ----------+---------+---------+---------+---------+---------+---------- 1080
     TACATGATCCTTATTTCCTGAAGGTAAGTGAACGTTCCGAAAACAAAAACTATTTCGTTC

338  C  T  R  N  K  G  L  P  F  T  C  K  A  F  V  F  D  K  A  R  357

1081 AAAACAATGCCTCTGGTTCCCCTTCAATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGG
     ----------+---------+---------+---------+---------+---------+---------- 1140
     TTTTGTTACGGAGACCAAGGGGAAGTTATCGTACAGTTCACCTCACTTTTTTCTTAAACC

358  K  Q  C  L  W  F  P  F  N  S  M  S  S  G  V  K  K  E  F  G  377

1141 CCATGAATTTGACCTCTATGAAAACAAAGACTACATTAGAAACTGCATCATTGGTAAAGG
     ----------+---------+---------+---------+---------+---------+---------- 1200
     GGTACTTAAACTGGAGATACTTTTGTTTCTGATGTAATCTTTGACGTAGTAACCATTTCC

378  H  E  F  D  L  Y  E  N  K  D  Y  I  R  N  C  I  I  G  K  G  397
```

(continued)

(continued)

```
                ACGCAGCTACAAGGGAACAGTATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGGAG
1201            ---------+---------+---------+---------+---------+---------+ 1260
                TGCGTCGATGTTCCCTTGTCATAGATAGTGATTCTCACCGTAGTTTACAGTCGGGACCTC

 398              R  S  Y  K  G  T  V  S  I  T  K  S  G  I  K  C  Q  P  W  S   417

                TTCCATGATACCACACGAACACAGCTATCGGGGTAAAGACCTACAGGAAAACTACTGTCG
1261            ---------+---------+---------+---------+---------+---------+ 1320
                AAGGTACTATGGTGTGCTTGTGTCGATAGCCCCATTTCTGGATGTCCTTTTGATGACAGC

 418              S  M  I  P  H  E  H  S  Y  R  G  K  D  L  Q  E  N  Y  C  R   437

                AAATCCTCGAGGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAGGTACGCTA
1321            ---------+---------+---------+---------+---------+---------+ 1380
                TTTAGGAGCTCCCCTTCTTCCCCCTGGGACCACAAAGTGTTCGTTAGGTCTCCATGCGAT

 438              N  P  R  G  E  E  G  G  P  W  C  F  T  S  N  P  E  V  R  Y   457

                CGAAGTCTGTGACATTCCTCAGTGTTCAGAAGTTGAATGCATGACCTGCAATGGGGAGAG
1381            ---------+---------+---------+---------+---------+---------+ 1440
                GCTTCAGACACTGTAAGGAGTCACAAGTCTTCAACTTACGTACTGGACGTTACCCCTCTC

 458              E  V  C  D  I  P  Q  C  S  E  V  E  C  M  T  C  N  G  E  S   477

                TTATCGAGGTCTCATGGATCATACAGAATCAGGCAAGATTTGTCAGCGCTGGGATCATCA
1441            ---------+---------+---------+---------+---------+---------+ 1500
                AATAGCTCCAGAGTACCTAGTATGTCTTAGTCCGTTCTAAACAGTCGCGACCCTAGTAGT

 478              Y  R  G  L  M  D  H  T  E  S  G  K  I  C  Q  R  W  D  H  Q   497

                GACACCACACCGGCACAAATTCTTGCCTGAAAGATATCCCGACAAGGGCTTTGATGATAA
1501            ---------+---------+---------+---------+---------+---------+ 1560
                CTGTGGTGTGGCCGTGTTTAAGAACGGACTTTCTATAGGGCTGTTCCCGAAACTACTATT

 498              T  P  H  R  H  K  F  L  P  E  R  Y  P  D  K  G  F  D  D  N   517

                TTATTGCCGCAATCCCGATGGCCAGCCGAGGCCATGGTGCTATACTCTTGACCCTCACAC
1561            ---------+---------+---------+---------+---------+---------+ 1620
                AATAACGGCGTTAGGGCTACCGGTCGGCTCCGGTACCACGATATGAGAACTGGGAGTGTG

 518              Y  C  R  N  P  D  G  Q  P  R  P  W  C  Y  T  L  D  P  H  T   537

                CCGCTGGGAGTACTGTGCAATTAAAACATGCGCTGACAAAGCTGACGACGACGACAAACA
1621            ---------+---------+---------+---------+---------+---------+ 1680
                GGCGACCCTCATGACACGTTAATTTTGTACGCGACTGTTTCGACTGCTGCTGCTGTTTGT

 538              R  W  E  Y  C  A  I  K  T  C  A  D  K  A  D  D  D  D  K  H   557

                CCACCACCACCACCACCACTAGGGTCGAC
1681            ---------+---------+---------  1709
                GGTGGTGGTGGTGGTGGTGATCCCAGCTG

 558              H  H  H  H  H  H  *           563
```

FIG 3a

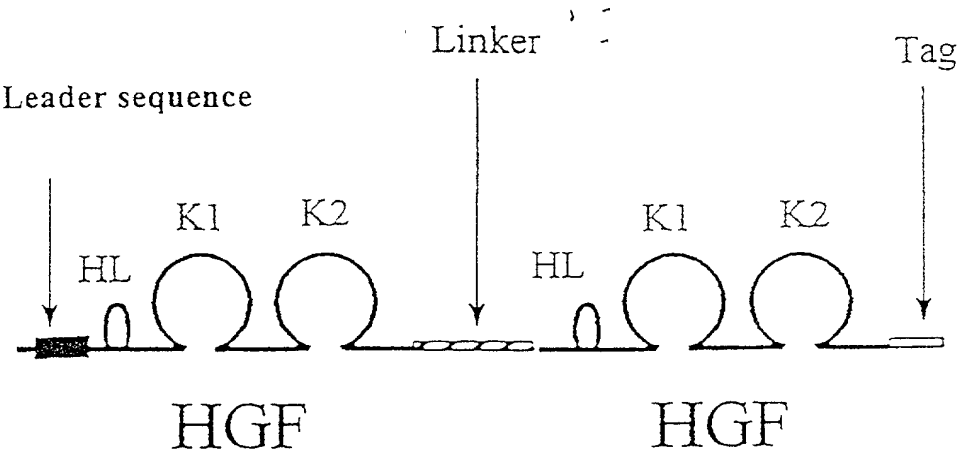

FIG 3b

```
1   GGATCCGCCAGCCCGTCCAGCAGCACCATGTGGGTGACCAAACTCCTGCCAGCCCTGCTG   60
    CCTAGGCGGTCGGGCAGGTCGTCGTGGTACACCCACTGGTTTGAGGACGGTCGGGACGAC

1                                   M  W  V  T  K  L  L  P  A  L  L   11

61  CTGCAGCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCATCCCCTATGCAGAGGGA   120
    GACGTCGTACAGGAGGACGTAGAGGAGGACGAGGGGTAGCGGTAGGGGATACGTCTCCCT

12  L  Q  H  V  L  L  H  L  L  L  L  P  I  A  I  P  Y  A  E  G   31

121 CAAAGGAAAAGAAGAAATACAATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAATC   180
    GTTTCCTTTTCTTCTTTATGTTAAGTACTTAAGTTTTTTAGTCGTTTCTGATGGGATTAG

32  Q  R  K  R  R  N  T  I  H  E  F  K  K  S  A  K  T  T  L  I   51

181 AAAATAGATCCAGCACTGAAGATAAAAACCAAAAAAGTGAATACTGCAGACCAATGTGCT   240
    TTTTATCTAGGTCGTGACTTCTATTTTTGGTTTTTTCACTTATGACGTCTGGTTACACGA

52  K  I  D  P  A  L  K  I  K  T  K  K  V  N  T  A  D  Q  C  A   71

241 AATAGATGTACTAGGAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTTTGATAAA   300
    TTATCTACATGATCCTTATTTCCTGAAGGTAAGTGAACGTTCCGAAAACAAAAACTATTT

72  N  R  C  T  R  N  K  G  L  P  F  T  C  K  A  F  V  F  D  K   91

301 GCAAGAAAACAATGCCTCTGGTTCCCCTTCAATAGCATGTCAAGTGGAGTGAAAAAAGAA   360
    CGTTCTTTTGTTACGGAGACCAAGGGGAAGTTATCGTACAGTTCACCTCACTTTTTTCTT

92  A  R  K  Q  C  L  W  F  P  F  N  S  M  S  S  G  V  K  K  E   111

361 TTTGGCCATGAATTTGACCTCTATGAAAACAAAGACTACATTAGAAACTGCATCATTGGT   420
    AAACCGGTACTTAAACTGGAGATACTTTTGTTTCTGATGTAATCTTTGACGTAGTAACCA

112 F  G  H  E  F  D  L  Y  E  N  K  D  Y  I  R  N  C  I  I  G   131

421 AAAGGACGCAGCTACAAGGGAACAGTATCTATCACTAAGAGTGGCATCAAATGTCAGCCC   480
    TTTCCTGCGTCGATGTTCCCTTGTCATAGATAGTGATTCTCACCGTAGTTTACAGTCGGG

132 K  G  R  S  Y  K  G  T  V  S  I  T  K  S  G  I  K  C  Q  P   151

481 TGGAGTTCCATGATACCACACGAACACAGCTATCGGGGTAAAGACCTACAGGAAAACTAC   540
    ACCTCAAGGTACTATGGTGTGCTTGTGTCGATAGCCCCATTTCTGGATGTCCTTTTGATG

152 W  S  S  M  I  P  H  E  H  S  Y  R  G  K  D  L  Q  E  N  Y   171
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(continued)

(continued)

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541  TGTCGAAATCCTCGAGGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAGGTA  600
     ACAGCTTTAGGAGCTCCCCTTCTTCCCCCTGGGACCACAAAGTGTTCGTTAGGTCTCCAT

172  C  R  N  P  R  G  E  E  G  G  P  W  C  F  T  S  N  P  E  V   191

601  CGCTACGAAGTCTGTGACATTCCTCAGTGTTCAGAAGTTGAATGCATGACCTGCAATGGG  660
     GCGATGCTTCAGACACTGTAAGGAGTCACAAGTCTTCAACTTACGTACTGGACGTTACCC

192  R  Y  E  V  C  D  I  P  Q  C  S  E  V  E  C  M  T  C  N  G   211

661  GAGAGTTATCGAGGTCTCATGGATCATACAGAATCAGGGAAGATTTGTCAGCGCTGGGAT  720
     CTCTCAATAGCTCCAGAGTACCTAGTATGTCTTAGTCCGTTCTAAACAGTCGCGACCCTA

212  E  S  Y  R  G  L  M  D  H  T  E  S  G  K  I  C  Q  R  W  D   231

721  CATCAGACACCACACCGGCACAAATTCTTGCCTGAAAGATATCCCGACAAGGGCTTTGAT  780
     GTAGTCTGTGGTGTGGCCGTGTTTAAGAACGGACTTTCTATAGGGCTGTTCCCGAAACTA

232  H  Q  T  P  H  R  H  K  F  L  P  E  R  Y  P  D  K  G  F  D   251

781  GATAATTATTGCCGCAATCCCGATGGCCAGCCGAGGCCATGGTGCTATACTCTTGACCCT  840
     CTATTAATAACGGCGTTAGGGCTACCGGTCGGCTCCGGTACCACGATATGAGAACTGGGA

252  D  N  Y  C  R  N  P  D  G  Q  P  R  P  W  C  Y  T  L  D  P   271

841  CACACCCGCTGGGAGTACTGTGCAATTAAAACATGCGCTGACAAAGCTTCGGGCGGTGGC  900
     GTGTGGGCGACCCTCATGACACGTTAATTTTGTACGCGACTGTTTCGAAGCCCGCCACCG

272  H  T  R  W  E  Y  C  A  I  K  T  C  A  D  K  A  S  G  G  G   291

901  GGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTCTAGAGGGACAAAGGAAAAGAAGA  960
     CCAAGACCACCGCCACCGAGGCCGCCACCGCCAAGAGATCTCCCTGTTTCCTTTTCTTCT

292  G  S  G  G  G  G  S  G  G  G  G  S  L  E  G  Q  R  K  R  R   311

961  AATACAATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAATCAAAATAGATCCAGCA  1020
     TTATGTTAAGTACTTAAGTTTTTTAGTCGTTTCTGATGGGATTAGTTTTATCTAGGTCGT

312  N  T  I  H  E  F  K  K  S  A  K  T  T  L  I  K  I  D  P  A   331

1021 CTGAAGATAAAAACCAAAAAAGTGAATACTGCAGACCAATGTGCTAATAGATGTACTAGG  1080
     GACTTCTATTTTTGGTTTTTTCACTTATGACGTCTGGTTACACGATTATCTACATGATCC

332  L  K  I  K  T  K  K  V  N  T  A  D  Q  C  A  N  R  C  T  R   351

1081 AATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTTTGATAAAGCAAGAAAACAATGC  1140
     TTATTTCCTGAAGGTAAGTGAACGTTCCGAAAACAAAAACTATTTCGTTCTTTTGTTACG

352  N  K  G  L  P  F  T  C  K  A  F  V  F  D  K  A  R  K  Q  C   371

1141 CTCTGGTTCCCCTTCAATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCATGAATTT  1200
     GAGACCAAGGGGAAGTTATCGTACAGTTCACCTCACTTTTTTCTTAAACCGGTACTTAAA

372  L  W  F  P  F  N  S  M  S  S  G  V  K  K  E  F  G  H  E  F   391
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(continued)

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1201 GACCTCTATGAAAACAAAGACTACATTAGAAACTGCATCATTGGTAAAGGACGCAGCTAC 1260
     CTGGAGATACTTTTGTTTCTGATGTAATCTTTGACGTAGTAACCATTTCCTGCGTCGATG
 392 D  L  Y  E  N  K  D  Y  I  R  N  C  I  I  G  K  G  R  S  Y   411

1261 AAGGGAACAGTATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGGAGTTCCATGATA 1320
     TTCCCTTGTCATAGATAGTGATTCTCACCGTAGTTTACAGTCGGGACCTCAAGGTACTAT
 412 K  G  T  V  S  I  T  K  S  G  I  K  C  Q  P  W  S  S  M  I   431

1321 CCACACGAACACAGCTATCGGGGTAAAGACCTACAGGAAAACTACTGTCGAAATCCTCGA 1380
     GGTGTGCTTGTGTCGATAGCCCCATTTCTGGATGTCCTTTTGATGACAGCTTTAGGAGCT
 432 P  H  E  H  S  Y  R  G  K  D  L  Q  E  N  Y  C  R  N  P  R   451

1381 GGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAGGTACGCTACGAAGTCTGT 1440
     CCCCTTCTTCCCCCTGGGACCACAAAGTGTTCGTTAGGTCTCCATGCGATGCTTCAGACA
 452 G  E  E  G  G  P  W  C  F  T  S  N  P  E  V  R  Y  E  V  C   471

1441 GACATTCCTCAGTGTTCAGAAGTTGAATGCATGACCTGCAATGGGGAGAGTTATCGAGGT 1500
     CTGTAAGGAGTCACAAGTCTTCAACTTACGTACTGGACGTTACCCCTCTCAATAGCTCCA
 472 D  I  P  Q  C  S  E  V  E  C  M  T  C  N  G  E  S  Y  R  G -   491

1501 CTCATGGATCATACAGAATCAGGCAAGATTTGTCAGCGCTGGGATCATCAGACACCACAC 1560
     GAGTACCTAGTATGTCTTAGTCCGTTCTAAACAGTCGCGACCCTAGTAGTCTGTGGTGTG
 492 L  M  D  H  T  E  S  G  K  I  C  Q  R  W  D  H  Q  T  P  H   511

1561 CGGCACAAATTCTTGCCTGAAAGATATCCCGACAAGGGCTTTGATGATAATTATTGCCGC 1620
     GCCGTGTTTAAGAACGGACTTTCTATAGGGCTGTTCCCGAAACTACTATTAATAACGGCG
 512 R  H  K  F  L  P  E  R  Y  P  D  K  G  F  D  D  N  Y  C  R   531

1621 AATCCCGATGGCCAGCCGAGGCCATGGTGCTATACTCTTGACCCTCACACCCGCTGGGAG 1680
     TTAGGGCTACCGGTCGGCTCCGGTACCACGATATGAGAACTGGGAGTGTGGGCGACCCTC
 532 N  P  D  G  Q  P  R  P  W  C  Y  T  L  D  P  H  T  R  W  E   551

1681 TACTGTGCAATTAAAACATGCGCTGACAAAGCTGACGACGACGACAAACACCACCACCAC 1740
     ATGACACGTTAATTTTGTACGCGACTGTTTCGACTGCTGCTGCTGTTTGTGGTGGTGGTG
 552 Y  C  A  I  K  T  C  A  D  K  A  D  D  D  D  K  H  H  H  H   571

1741 CACCACCACTAGGGTCGAC 1759
     GTGGTGGTGATCCCAGCTG
 572 H  H  H  *           574
```

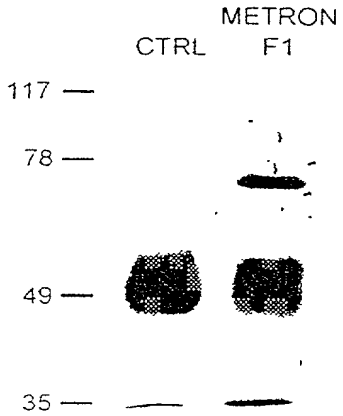


Fig 4

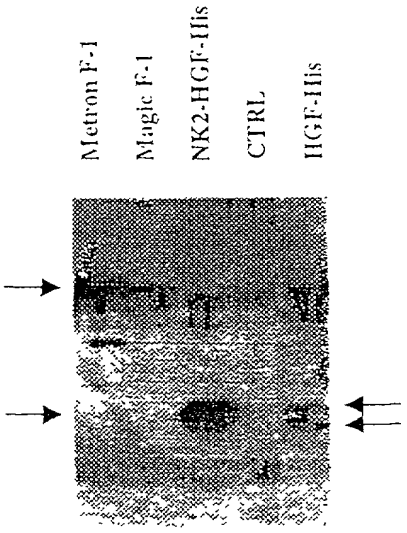


Fig 5A

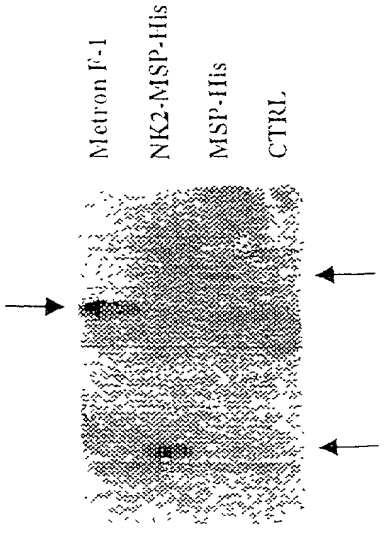


Fig 5B

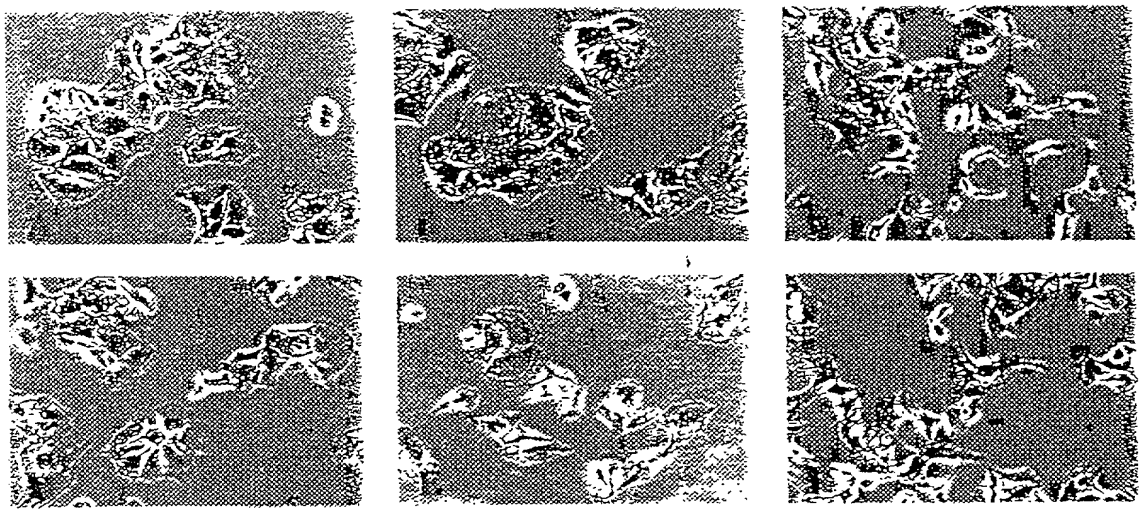


Fig 6

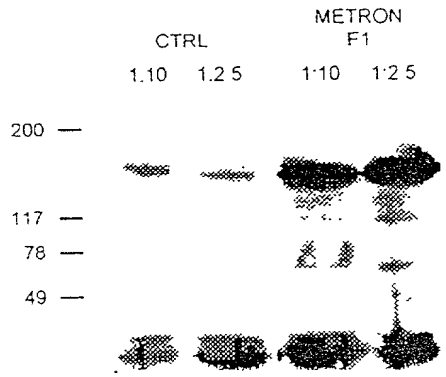
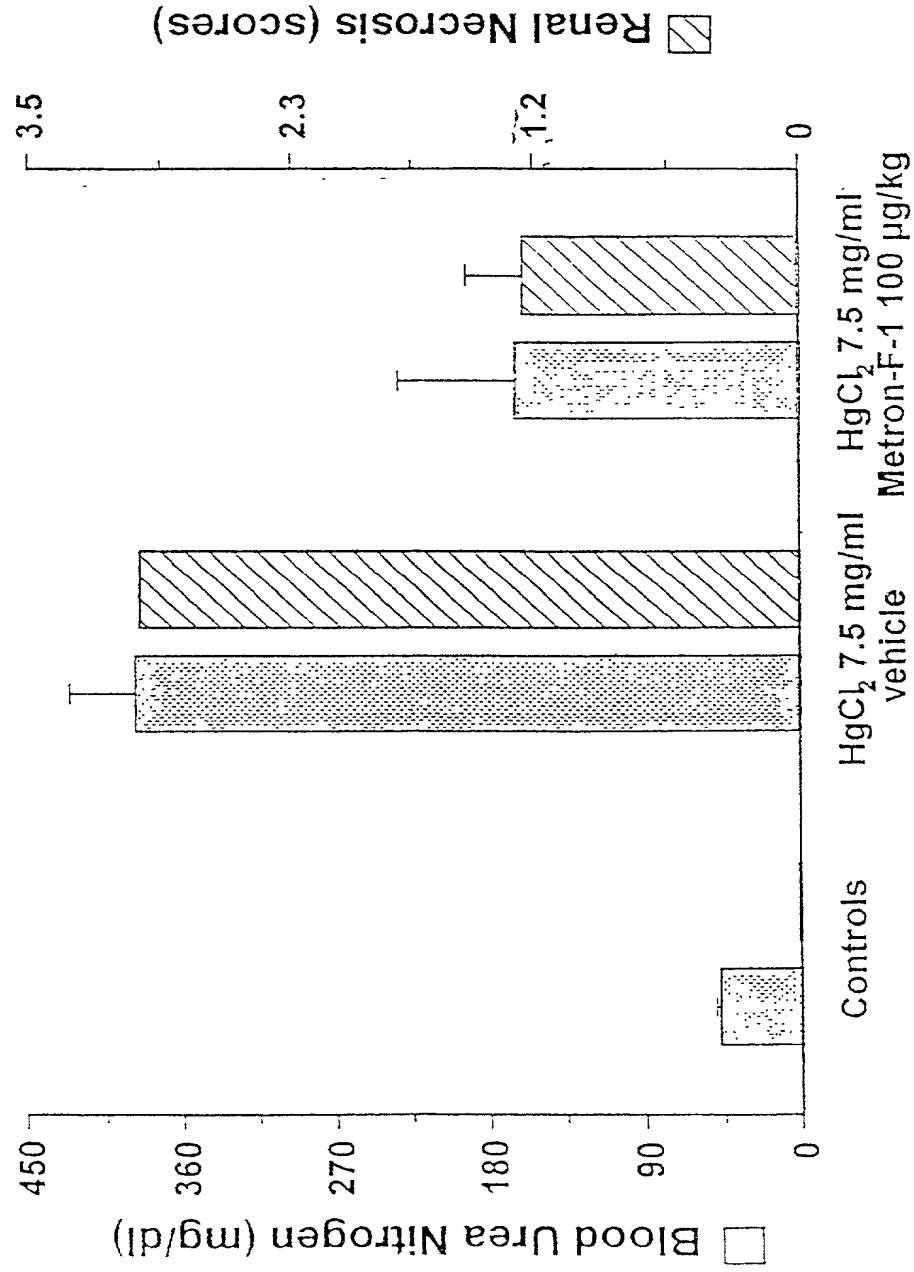


Fig 7

FIG 8


Renal Necrosis (scores)
3.5
2.3
1.2
0
Blood Urea Nitrogen (mg/dl)
450
360
270
180
90
0
Controls
HgCl2 7.5 mg/ml vehicle
HgCl2 7.5 mg/ml Metron-F-1 100 µg/kg

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PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title: Recombinant proteins derived from HGF and MSP

Fill in Appropriate Information - For Use Without Specification Attached:

the specification of which is attached hereto. If not attached hereto,
the specification was filed on _____ as
United States Application Number _____;
and amended on _____ (if applicable) and/or
the specification was filed on 27.01.1999 as PCT
International Application Number PCT/EP99/00478; and was
amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information: (if appropriate)

Prior Foreign Application(s)			Priority Claimed	
(Number)	(Country)	(Month/Day/Year Filed)		
MI98A000179	Italy	30.01.1998	☒ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below.

Insert Provisional Application(s): (if any)

(Application Number)	(Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Insert Requested Information: (if appropriate)

Country	Application Number	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Insert Prior U.S. Application(s): (if any)

(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)

Attorney Docket No.:

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full Name of First or Sole Inventor: Insert Name of Inventor

Insert Date This Document is Signed

Insert Residence Insert Citizenship

Insert Post Office Address

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Full Name of Fifth Inventor, if any: see above

*DATE OF SIGNATURE